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UNIVERSITY OF ALBERTA

THE EFFECTS OF SEROTONIN DISRUPTION IN TWO ANIMAL MODELS OF ANXIETY.

BY

(C)

ANGELA LYNNE ROBINSON

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

DEPARTMENT OF PSYCHOLOGY

Edmonton, Alberta

Spring, 1992.

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The effects of serotonin disruption in two animal models of anxiety" submitted by Angela Lynne Robinson in partial fulfilment of the requirements for the degree of Master of Science.

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March 4, 1992.

Abstract

Several researchers have attempted to determine whether the inhibition of serotonin results in an inhibition of 'anxiety' in humans and lower animals. However, the results of this research have been inconsistent and inconclusive. In this thesis, the elevated plus-maze test and the shock-probe burying test of anxiolysis were used in combination to investigate the effects of serotonin disruption in rats. In Experiment 1, p-CPA (a serotonin synthesis inhibitor) produced a significant anxiolytic-like increase in open-arm activity in the plus-maze test, and a non-significant anxiolytic-like decrease in burying behaviour in the shock-probe test, compared to saline-injected control animals. In Experiment 2, a similar pattern of behavioral results emerged in animals with combined electrolytic lesions of the dorsal and median raphe nuclei, compared to animals receiving sham-lesions. In Experiment 3, selective lesions of the dorsal raphe produced some anxiolytic-like effects in the plus-maze test, but no evidence of anxiolysis in the shock-probe test, and lesions of the median raphe produced no evidence of anxiolysis in either behavioral test, compared to sham-lesioned animals. In Experiment 4, lesions of either the dorsal raphe or the posterior septum produced significant anxiolytic-like behaviour in the plus-maze and shock-probe tests compared to sham-lesioned animals. The results of these four experiments provide some evidence that serotonin disruption can produce anxiolysis in animals.

However, the inconsistency of results in the plus-maze test and the shock-probe test emphasizes the need for further research into the role of serotonin in anxiety.

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List of Symbols

5-HT..... 5-hydroxytryptamine

5-HTP..... 5-hydroxytryptophan

5,6-DHT.... 5,6-dihydroxytryptamine

5,7-DHT.... 5,7-dihydroxytryptamine

8-OH-DPAT.. 8-hydroxy-2-(di-*N*-propylamino)tetralin

ACh..... acetylcholine

BZ..... benzodiazepine

DA..... dopamine

DR..... dorsal raphe

MR..... median raphe

NE..... norepinepherine

p-CPA..... para-chlorophenylalanine

PTZ..... pentylenetetrazol

INTRODUCTION

Serotonin (5-hydroxytryptamine, or 5-HT) was originally isolated in 1948, when researchers identified a vaso-constricting compound in blood serum (Rapport, Green, & Page, 1948). It was later discovered that 5-HT could also be found in the mammalian central nervous system (Twarog & Page, 1953), and that there was regional variation of 5-HT concentration in the brain (Amin, Crawford, & Gaddum, 1954), suggesting that 5-HT might function as a neurotransmitter in the brain.

Since the discovery of brain 5-HT, a great deal of research has been directed toward the neuroanatomical, neuropharmacological, and neurobehavioral aspects of 5-HT-containing neurons. Serotonin cell bodies were localized, using formaldehyde-induced histofluorescence techniques, to a few nuclear groups positioned along the midline of the brain stem, from the medulla to the midbrain (Dahlstrom & Fuxe, 1964). These nuclear groups have been coded B1 (in the medulla) through B9 (in the tegmentum), and are commonly called the raphe nuclei. The 5-HT cells in the medulla generally project to the gray matter of the spinal cord, while pontine and tegmentum 5-HT cell regions project to the cerebellum and forebrain (Consolazione & Cuello, 1982). Two midbrain raphe nuclei, the B7 or dorsal raphe (DR) group, and the B8 or median raphe (MR) group, contain cell bodies

which project to forebrain structures. Projections from the DR and MR follow a variety of ascending paths (Consolazione et al., 1982), innervating numerous structures, including the hippocampus, amygdala, habenula, septum, olfactory bulbs, neocortex, striatal portions of the basal ganglia, and substantia nigra. The DR and MR jointly innervate most of these forebrain structures, though the density of DR projections to any structure usually differs from that of projections from the MR (Azmitia & Segal, 1978; Molliver, 1987). In addition to these connections, it appears the DR and MR are reciprocally connected to each other, perhaps modifying the others activity in the brain (Azmitia & Segal, 1978; Parent, Descarries, & Beaudet, 1981).

Pharmacologically, 5-HT appears to interact with many different types of 5-HT receptors in the mammalian brain. Originally, 5-HT receptors were classified as type "M" and type "D" receptors, and were later re-classified as type "1" and type "2" receptors (Wang & Peroutka, 1988). More recently, receptors have been classified into type "1", type "2", and type "3" receptors (Bradley et al, 1976).

While 5-HT binds to all 5-HT receptors, certain serotonergic drugs were found that bind preferentially to particular types of 5-HT receptors. Different serotonergic drugs often bind to different subclasses of 5-HT receptors (Leysen, 1985). Certain 5-HT receptors appear to mediate the inhibition of 5-HT systems. Presynaptic autoreceptors

are located on cell bodies and on nerve terminals of 5-HT neurons (Aghajanian & Haigler, 1974). Agonists of pre-synaptic 5-HT_{1A} autoreceptors stimulate the receptor, which in turn inhibits 5-HT transmission to the forebrain (Aghajanian & Haigler, 1974). Activation of post-synaptic 5-HT receptors (including post-synaptic 5-HT_{1A} receptors) appears to modulate the neural systems involved in a variety of behavioral and physiological processes.

The behaviours and physiological processes modulated by 5-HT are numerous and varied. Serotonergic systems have been implicated in modulating locomotor activity levels, thermoregulation, sexual behaviour (Hillegaart, 1991), pain sensitivity, sleep-waking cycles (Seiden & Dykstra, 1977), aggressive behaviours (Jacobs & Cohen, 1976), food intake (Bendotti & Samanin, 1986; Bendotti & Samanin, 1987; Fletcher & Davies, 1990), drinking (Barofski, Grier, & Pradhan, 1980), and anxiety (Iversen, 1984).

Serotonin and anxiety. The role of 5-HT in mediating anxiety-related behaviours in mammals is a topic which has been explored extensively by a number of researchers, and is the focus of the present research. In order to summarize what is known regarding the role of 5-HT in the control of anxiety-related behaviours, the relevant literature will be reviewed in the first part of this introduction. Much of this literature suggests that reduced 5-HT function is

correlated with reduced anxiety-related behaviours in animals. However, there have been several investigations which have failed to find this correlation. Possible reasons for these inconsistent findings will be discussed in the second part of the introduction. Finally, the rationale and purpose of the present research will be presented.

Animal models of anxiety. In order to experimentally study the role of 5-HT in animal "anxiety", we must first develop a model of animal anxiety. Although it cannot be shown that such models reflect a state of anxiety in animals similar to that of human anxiety, exposure to (or threat of) an aversive stimulus can produce fear reactions in both humans and infrahumans. In general, animal models of anxiety involve some aversive stimulus (eg., electric foot-shock), and a behaviour that is reliably elicited by the aversive stimulus (eg., running away). Thus, one criterion for an animal model of anxiety is that the model should reflect some features of situations producing anxiety-related responses in humans, such as uncertainty or the threat of a painful stimulus. A second criterion for such animal models is the sensitivity of the model to manipulations or treatments which reduce human anxiety, such as a sensitivity to standard anxiolytic drugs (e.g., benzodiazepines [BZ]). Administration of such drugs should produce a reliable, dose-dependent reduction in the index of anxiety in the

animal model. The model should also be relatively insensitive to non-anxiolytic drugs, such as stimulants or neuroleptics. Finally, the model should provide some physiological evidence of stress or fear also seen in anxious humans, such as an elevated heart rate, increased blood corticosteroid levels, or adrenaline release (for further discussion of these criteria, see Lister, 1990). Although these criteria are strictly correlational, they can guide researchers towards appropriate experimental analogs for studying the neurobiological bases of anxiety (Lal & Emmett-Oglesby, 1983).

The conflict model. Evidence suggesting 5-HT may be involved in anxiety arises mainly from research utilizing the "conflict" model of anxiety. The conflict paradigm was originally developed by Geller and Seifter (1960) as an objective means of assessing "neurotic" behaviours in rats. Since that time, modifications of the original conflict test have been developed, including the Vogel conflict test (Vogel, Beer, & Clody, 1971). Conflict tests are among the most widely utilized animal models of anxiety (File, 1987; Lal & Emmet-Oglesby, 1983).

In "conflict" paradigms, food- or water-deprived animals are first trained to perform an operant behaviour (e.g., a bar press) to obtain a food or fluid reward. Once the behaviour is firmly established, signalled periods of

punished responding are introduced, where an aversive consequence (usually electric foot-shock) is paired with the reward-producing behaviour, presumably causing conflict between the animal's motivations to obtain food and to avoid shock. During these periods of signalled punished responding, animals generally show a suppression of responding compared to response levels during non-punished periods. Clinically effective anxiolytic drugs, (e.g., BZs) antagonize this suppressed responding under the "conflict" contingency. In other words, animals begin to respond more frequently despite the aversive consequences of obtaining reward during the signalled shock period. This "anti-conflict" effect appears to be specific to anxiolytic drugs, as drugs from other therapeutic classes (e.g., neuroleptics, amphetamines) do not produce a robust anti-conflict effect (Stein, Wise & Belluzzi, 1975; Cook & Davidson, 1973; Geller & Seifter, 1960; Sepinwall & Cook, 1975; Vogel et al., 1971).

5-HT neurotoxins and conflict behaviour. The conflict paradigm has been used to investigate the role of 5-HT systems in anxiety and anxiolytic drug action. Several lines of research suggest that reductions of 5-HT function can produce anti-conflict effects similar to those of anxiolytic drugs. For example, neurotoxic lesions of 5-HT cell bodies using intracerebroventricular (ICV) 5,6-

dihydroxytryptamine (5,6-DHT) or 5,7-dihydroxytryptamine (5,7-DHT) produced significant anticonflict effects in rats (Commissaris, Lyness & Rech, 1981; Soderpalm & Engel, 1991; Stein et al., 1975). More localized 5,7-DHT lesions in the tegmentum of the rat brain (Tye, Everitt, & Iversen, 1977) also produced anti-conflict effects in rats. Since the major 5-HT cell body regions projecting to the forebrain (the DR and the MR) lie in the tegmentum, these results suggest these cell regions are important in mediating anxiety in animals. More selective 5,6-DHT lesions of the DR alone were also shown to produce anti-conflict effects in rats (Green & Hodges, 1986). Similar effects were found with lesions of the DR using 5,7-DHT (Thiebot, Hamon, & Soubrie, 1983). It is important to note that these neurotoxic lesions increased responding specifically during the signalled punishment periods. Responding during the unpunished periods of the conflict test remained unaffected by such lesions (Thiebot et al, 1983). It thus appears that research utilizing neurotoxic 5-HT lesions supports the idea that impairment of 5-HT function leads to selective anxiolytic-like effects in animals.

<u>p-CPA and conflict behaviour.</u> Systemic administration of the 5-HT synthesis inhibitor, para-chlorophenylalanine (p-CPA) is another method of reducing 5-HT function in the rat brain (Koe & Weissman, 1966). In tests using the conflict

paradigm, p-CPA-induced 5-HT depletion generally produced anti-conflict effects in rats (Geller & Blum, 1970; Green, Hodges, & Summerfield, 1983; Hartmann & Geller, 1971; Petersen & Lassen, 1981; Robichaud & Sledge, 1969; Shephard, Buxton, & Broadhurst, 1982; Soderpalm & Engel, 1989; Stevens, Fechter & Resnick, 1969; Tye, Iversen, & Green, 1979) in a manner similar to that of BZ drugs (Stein, Wise & Berger, 1973; Wise, Berger & Stein, 1972). Systemic administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP), was effective in reversing the anti-conflict effect of p-CPA administration (Geller & Blum, 1970), further suggesting p-CPA produces anti-conflict effects through reduction of 5-HT function.

5-HT antagonists and conflict behaviour. A third method of reducing 5-HT function is by administration of 5-HT receptor antagonists such as methylsergide or cyproheptadine. Graeff and Schoenfeld (1970) discovered that the 5-HT antagonists methylsergide and bromolysergic acid produced an increase in punished responding (i.e., an anticonflict effect) in pigeons. The anti-conflict effects of methylsergide have been replicated in rats by several researchers (Cook & Sepinwall, 1975; Graeff, 1974; Stein et al., 1975; Winter, 1972). The neuropharmacological specificity of this effect was tested by comparing the effects of the 5-HT antagonist, methylsergide, with the

effects of norepinepherine (NE) antagonists, phentolamine and propranolol (Stein et al., 1975). Methylsergide produced an increase in punished responding in the conflict test, while NE antagonists suppressed responding in all phases of the conflict test (i.e., both punished and non-punished responding). These results support the hypothesis that reductions in 5-HT function mediate anti-conflict effects, while reductions in NE function do not (Stein et al., 1975). Anti-conflict effects have also been produced with other 5-HT antagonists, such as cinanserin (Cook & Sepinwall, 1975; Geller, Hartmann, Croy, & Haber, 1974; Kilts, Commissaris, Cordon, & Rech, 1982), and cyproheptadine (Graeff, 1974; Schoenfeld, 1976), further suggesting 5-HT disruption is central to producing anti-conflict effects.

5-HT, benzodiazepine drugs, and conflict behaviour. A separate, but related line of research investigated whether 5-HT might play a role in mediating the effects of standard anxiolytic drugs in the conflict test. The BZ class of anxiolytic drugs has been shown to reduce the turnover of several neurotransmitters, including dopamine (DA) and norepinepherine (NE; Koe & Weissman, 1966), as well as serotonin (Chase, Katz, & Kopin, 1970; Wise et al., 1972). Tolerance to the reduction in NE turnover developed rapidly, as did tolerance to the sedative effects of BZ drugs. In

contrast, reductions in 5-HT turnover persisted over repeated doses, as did the anti-conflict effects produced by BZ anxiolytic drugs. From these data, it was hypothesized that BZ drugs induce anxiolytic effects by reducing 5-HT turnover in the brain (Wise et al., 1972). Several other studies suggest 5-HT may mediate the anticonflict effects of BZ drugs. Direct injection of the BZ chlordiazepoxide into the DR produced anti-conflict effects (Theibot, Hamon, & Soubrie, 1982), while injections of 5-HT directly into the ventricles of the rat brain reversed the anti-conflict effect of BZ drugs (Wise et al., 1972). From this research, it appears that the anticonflict effect of BZ drugs may be mediated through a reduction of brain 5-HT function, particularly in the DR.

<u>5-HT_{1A} agonists and conflict behaviour.</u> Recently, interest in the role of 5-HT in anxiety has been rekindled with the discovery of 5-HT receptor sub-types, particularly the 5-HT_{1A} sub-type (Bradley et al.,1986). Interestingly, agonists of the 5-HT_{1A} receptor include the clinically active anti-anxiety agents, buspirone and gepirone. In clinical trials, these 5-HT_{1A} agonists appear to be as effective as BZ drugs in reducing patient anxiety, with fewer side-effects (Csanalosi, Schweizer, Case, & Rickels, 1987; Goa & Ward, 1986; Taylor, Eison, Riblet, & Vandermaelen, 1985). The anxiolytic action of 5-HT_{1A}

agonists is thought to be a function of autoreceptor site activation, producing a net reduction of 5-HT function in the brain (Aghajanian & Wang, 1978). In support of this idea, buspirone was shown to inhibit firing rates of DR neurons in vivo (Vandermaelen, Matheson, Wilderman, & Patterson, 1986).

The effects of 5-HT_{1A} agonists have been tested in the conflict paradigm. Systemic injections of buspirone and the experimental 5-HT_{1A} agonist 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT), both produced anti-conflict effects in modified Vogel water-lick conflict tests (Carli & Samanin 1988; Engel, Hjorth, Svensson, Carlsson, & Liljequist, 1984; Moser et al., 1990). Further research showed that direct infusion of buspirone, ipsapirone, and 8-OH-DPAT, into the DR produced typical anti-conflict effects in rats (Higgens, Bradbury, Jones, & Oakley, 1988; Higgens, Jones, & Oakley, 1987). The results of this latter research suggest that the DR may mediate anti-conflict effects of 5-HT_{1A} agonists.

Problems with conflict test research. The research previously discussed in this introduction suggests that inhibition of 5-HT function by any one of a number of methods invariably produces anti-conflict effects in animals. However, there are a number of cases where this relationship between 5-HT and experimental anxiety has not

been found. For example, in some studies, 5,7-DHT lesions of the DR did not alter suppressed responding in rats, nor did such lesions alter the ability of BZ drugs to produce anti-conflict effects (Commissaris, Lyness, & Rech, 1981; Thiebot, Hamon & Soubrie, 1982; Thiebot, Soubrie, Hamon, & Simon, 1984). Depletion of brain 5-HT using p-CPA has also failed to produce anti-conflict effects in one study (Blakely & Parker, 1973). There are reports that 5-HT receptor blockade using methylsergide did not produce anti-conflict effects (Kilts, Commissaris, & Rech, 1981; Petersen & Lassen, 1981). Receptor blockade using the 5-HT antagonists cinanserin (Peterson & Lassen, 1981; Winter, 1972) and cyproheptadine (Petersen & Lassen, 1981) has also failed to alter conflict behaviour in some instances. Thus, there are a number of studies which contradict the notion that reducing 5-HT function invariably produces anti-conflict effects.

Furthermore, there is evidence that BZ drugs do not act exclusively through 5-HT systems to produce their anti-conflict effects. Investigators studying the interactions between 5-HT manipulations and BZ drugs have occasionally found that neither 5-HT antagonists nor p-CPA treatment altered the effectiveness of BZ administration in producing anti-conflict effects (Commissaris & Rech, 1982; Kilts et al., 1982; Shephard et al., 1982). Conversely, other research has shown that 5-HT lesions and BZ drug treatment

in combination produced an additive anti-conflict effect (Tye et al., 1977, for an overview of procedures and results presented thus far, see Table 1) .

These conflicting results do not, however, entirely invalidate the hypothesis that 5-HT reduction produces anxiolysis. There could be a number of reasons why a reduction in 5-HT function might not lead to clear anti-conflict effects. One reason might be related to variations in lesion or drug parameters across experiments. If different serotonergic sites were lesioned, or if different doses of a particular serotonergic drug were used, we might not expect to find equivalent behavioral effects in the conflict test. For example, large doses (1-18 mg/kg) of methylsergide failed to produce anti-conflict effects (Kilts et al., 1981; Kilts, 1982), while lower doses (0.01-1 mg/kg) produced such anti-conflict effects (Graeff & Schoenfeld, 1970). However, there are cases (Table 1) where methylsergide dose parameters were equivalent, yet inconsistent behavioral effects in the conflict test were found (e.g., Kilts et al., 1982 and Winter, 1972). Other instances where similar drug doses produced inconsistent effects in the conflict test, can also be found in studies using cyproheptadine (e.g., Kilts et al., 1982; Schoenfeld, 1976), cinanserin (Geller et al., 1974; Petersen & Lassen, 1981) and buspirone (Gardner, 1986; Moser et al., 1990). At present, it is difficult to explain these inconsistencies

entirely on the basis of drug-dose parameters. Similarly, the neurotoxic lesion literature shows no consistent pattern of anti-conflict effects that can be correlated with particular parameters, such as dose of neurotoxin, or route of administration (see Table 1).

In addition, there are instances where a considerable amount of variation in pharmacological parameters (e.g., dose, route of drug administration, injection-test interval, and number of drug injections) did not influence behavioral effects in the conflict test. For example, researchers utilizing p-CPA have used a variety of administration schedules (e.g., Blakely et al, 1973, Geller & Blum, 1970, Green et al., 1983, and Robichaud & Sledge, 1969) and administration routes (e.g., Petersen & Lassen, 1981 and Robichaud & Sledge, 1969), in combination with a variety of dose parameters (e.g., Geller & Blum, 1970; Green et al., 1983). Despite this variability, p-CPA generally produced reliable anti-conflict effects (see Table 1). Taken together, these observations suggest that factors other than simple pharmacological parameters account for the variability in the effects of 5-HT manipulations on conflict behaviour.

One such factor might be the selectivity of the various 5-HT manipulations for affecting 5-HT function. It is possible that inconsistencies in the anti-conflict effects of any given 5-HT manipulation may be due to secondary

effects on other neurotransmitter systems. Even relatively selective manipulations such as serotonergic cell lesions using 5,7-DHT, or 5-HT depletion using p-CPA, appear to affect other neurotransmitters in the brain, such as NE and DA (Koe & Weissman, 1966). The selectivity of 5-HT receptor antagonists such as cyproheptadine is also not absolute, since some of these agents appear to inhibit acetylcholine (Ach) as well as histamine (Ketelaars & Bruinvels, 1989). In such cases, it is difficult to exclude the possibility that secondary alteration of other neurotransmitters may have masked or altered the effects of 5-HT disruption on conflict behaviour.

Another factor to consider when interpreting the effects of 5-HT manipulations on anti-conflict behaviour is the effect of such manipulations on other 5-HT mediated behaviours. Manipulations of 5-HT systems are known to affect activity levels, feeding, drinking, sleeping, and pain sensitivity (e.g. 's, Bendotti & Samanin, 1986; Seiden & Dykstra, 1977). Changes in these behaviours could produce behavioral effects which might mask, or alter, anti-conflict effects, independently of specific effects of 5-HT on experimental anxiety. This is especially problematic for the conflict test, as this test involves food motivation, shock motivation, operant behaviour, as well as the ability of the rat to discriminate between punished and non-punished periods, all of which could be affected by 5-HT depletion.

There is ample evidence showing 5-HT reduction increases the consumption of food (Blundell & Latham, 1979; Bendotti & Samanin, 1986; Bendotti & Samanin, 1987; Fletcher & Davies, 1990) and fluid (Barofski et al., 1980) in rats. Additionally, p-CPA administration appears to increase the palatability of a sucrose fluid reinforcer (O'Hare, Tierney, & Shephard, 1991). Considering this evidence, we might ask whether the "anti-conflict" effects of 5-HT manipulations are due to reduced "anxiety", or to increased food or fluid motivation.

A number of studies have also shown that reduced 5-HT function in the brain is associated with increased pain sensitivity (e.g., Beitz, 1990). Serotonin systems, especially in the DR, may inhibit firing of cells that respond to painful exteroceptive stimulation (Prieto-Gomez, Dafney, and Reyez-Vazquez, 1989). If experimental 5-HT manipulations increase pain sensitivity by inhibiting 5-HT transmission, it is possible that anti-conflict effects could be masked by disruption of 5-HT function, especially in the raphe nuclei. In this case, animals may be less "anxious", but be more sensitive to shock.

There is also evidence suggesting 5-HT manipulations alter an animal's ability to learn appropriate responses to painful stimuli, independently of changes to the animal's pain sensitivity. Animals with raphe lesions showed delayed learning of a passive shock avoidance task, despite showing

normal reaction latencies on a hot plate test of pain sensitivity (Hole & Lorens, 1974). In this case, anti-conflict effects might be mimicked by a failure of the animal to recall an appropriate response to shock.

Another problem inherent in the conflict test is the relatively complex training requirements of this task, and the necessity for rats to discriminate between shock and non-shock periods. Any impairment in discriminative ability due to 5-HT manipulations would certainly complicate the interpretation of anti-conflict effects. There is some evidence suggesting lesions of the MR impair rats' performance on a T-maze brightness discrimination task (Wirtshafter, Montana, and Asin, 1983). It is therefore possible that in certain cases, anti-conflict effects might be produced through a secondary effect of a rat's failure to discriminate shock from non-shock periods.

Finally, it has been argued (Soubrie, 1986) that the apparent "anti-conflict" effects of 5-HT manipulations might reflect an impaired ability of rats to inhibit responding in general, instead of reflecting a specific, anxiolytic-induced disinhibition of punished responding. Since response inhibition is the index of anxiety in the conflict test, it is difficult to discriminate between these two alternative interpretations.

Many of the interpretational problems previously discussed arise as a result of the behavioral complexity of

the conflict test. The conflict test involves processes such as food motivation, pain sensitivity, discrimination, and learning capacity, which could all be influenced by 5-HT reduction in the brain. Therefore, it may not be surprising that reductions in 5-HT do not always result in clear anti-conflict effects. For this reason, the conflict test may not be the most sensitive method for investigating the role of 5-HT in anxiety.

Other models of anxiety. An alternative approach to studying the role of 5-HT in anxiety is to utilize animal models of anxiety based on simpler, naturally occurring adaptations to aversive or threatening stimuli. Bolles (1970) has argued that avoidance learning based on "species-specific" defense reactions is more readily acquired by animals than learning based on behaviours (e.g., bar pressing) that are not in the animals' natural repertoire of defensive responses. Additionally, Seligman (1971) suggested animals are naturally "prepared" to associate certain "non-arbitrary" combinations of stimuli and responses, and that such associations are acquired much faster than those between more "arbitrary" combinations of stimuli and responses. For example, animals readily associate a novel taste with sickness, even after only one pairing of the taste with sickness. However, animals do not as readily associate visual cues with illness (Seligman,

1971). Accordingly, an animal model of anxiety based on "prepared" adaptations should require little or no training, and would be more closely related to mechanisms ordinarily employed by animals to cope with aversive stimuli in their environment.

The plus-maze test. Several novel tests of animal anxiety based on the "prepared" fear reactions of animals have been developed in recent years (Lister, 1990; Treit, 1985a). One such test is the "elevated plus-maze" model (Pellow, File, Chopin, & Briley, 1985). In this test, animals are allowed to explore a novel, elevated four-arm maze for five minutes. Two of the maze arms are enclosed with walls, and the remaining two arms are open, with no walls. Rats tend to avoid the open arms of the maze, restricting most of their activity to the closed maze arms. If rats are confined to the open arms by the experimenter, physiological signs of stress (increased plasma corticosterone levels) as well as behavioral signs of stress (freezing, immobility, and defecation) are observed (Pellow et al., 1985). While rats normally avoid the open maze arms, administration of standard anxiolytic drugs (benzodiazepines, barbiturates), will decrease open-arm avoidance (measured as a percent increase in time in the open arms, and a percent increase in entries into the open arms). This increase in open arm activity defines an

"anxiolytic" response in the plus-maze. Interestingly, drugs thought to increase anxiety in humans (pentylenetetrazol [PTZ]) generally reduce open-arm activity below that observed in non-drugged rats (Pellow et al., 1985). Finally, the plus-maze is relatively insensitive to classes of drugs other than anxiolytics or anxiogenics (Pellow et al., 1985). Thus, the plus-maze provides a simple test of anxious behaviour in rats without using food or fluid rewards, or prior training of a behaviour.

The shock-probe test. A second novel animal model of anxiety is the shock-probe burying test (Treit, Pinel, & Fibiger, 1981). In this test, rats are allowed to explore an electrified probe attached to one end of a Plexiglas chamber. After contacting the probe (thus receiving a shock) rats often attempt to "bury" the probe with bedding material from the floor of the chamber with rapid, alternating forepaw thrusts. The shock-probe test requires no training and does not involve food or fluid rewards. Burying behaviour is also sensitive to standard anxiolytic drugs. Administration of BZ drugs (e.g., chlordiazepoxide), but not other classes of drugs (e.g., Beardsley, Papadakis, Fontana, & Commissaris, 1990) reduced the amount of time animals spent burying the probe (Blampied & Kirk, 1983; Treit et al., 1981), and increased the amount of time spent exploring the probe (Meert & Colpaert, 1986; Treit, 1990).

Chlordiazepoxide was also shown to abolish the shock-induced elevations in plasma corticosterone and adrenaline that occurs during the shock-probe test (De Boer, Slangen, & Van Der Gugten, 1990). The suppression of burying induced by chlordiazepoxide was reversed using BZ antagonists such as flumazenil (Treit, 1987). Additionally, the putative anxiogenic agents yohimbine and the beta-carboline, beta-CCE, increased the amount of time rats spent burying the probe (Tsuda, Ida, & Tanaka, 1988; Tsuda, Ida, Nishimura, & Tanaka, 1889). The inhibition of burying produced by BZs in the shock-probe test does not appear to be a result of behavioral sedation (Treit et al, 1981; Treit & Pesold, 1990), an impairment in associating shock with the probe (Blampied & Kirk, 1983), or analgesia (Treit, 1985b). Like the plus-maze, the shock-probe test appears to satisfy the criteria for an animal model of anxiety, and is less complex than the conflict test.

5-HT and simple models of anxiety. The plus-maze and shock-probe tests have also been used to study the effects of serotonergic drugs on animal anxiety. Although there have been exceptions (e.g., Moser, 1989), low doses of the 5-HT_{1A} agonist, buspirone, have produced anxiolytic effects in the plus-maze test (Dunn, Corbett, & Fielding, 1989; Kostowski, Plaznik, & Stefanski, 1989; Soderpalm, Hjorth, & Engel, 1989) and in the shock-probe test (Treit & Fundytus,

1988). Ipsapirone, another 5-HT_{1A} agonist, also produced anxiolytic effects in the plus-maze test (Critchley & Handley, 1987; Soderpalm et al., 1989), and in the shock-probe test (Korte & Bohus, 1990). Finally, whole-brain lesions of 5-HT cells produced by ICV infusion of 5,7-DHT produced an increase in open arm activity in the plus-maze test (Briley, Chopin, & Moret, 1990, Soderpalm & Engel, 1990).

Thesis rationale. Taken together, these behavioral, pharmacological, and physiological data suggest the plus-maze and the shock-probe tests may be useful for further studying the role of 5-HT in animal "anxiety". In addition to their simplicity, the combined use of these two tests is especially important. In the plus maze test, passive avoidance of novel, elevated platforms is the index of "anxiety" while in the shock-probe test, the index of "anxiety" is the active burying of the shock-source. Furthermore, an anxiolytic profile in the plus-maze test is indicated by an increase in a specific behaviour (open-arm activity), while in the shock-probe test, an anxiolytic profile is indicated by a decrease in a specific behaviour (burying the probe). For this reason, anxiolytic profiles seen in both tests after 5-HT manipulations would be difficult to explain in terms of non-specific effects on general activity, arousal, pain sensitivity, or behavioral

inhibition. If reductions in 5-HT function do indeed produce specific reductions in anxiety, then a specific pattern of results should emerge in both of these tests after 5-HT depletion; i.e., 5-HT depleted rats should show increases in open arm activity in the plus-maze test, and decreases in burying behaviour in the shock-probe test.

Thus, the purpose of the present thesis was to further evaluate the role of 5-HT in anxiety, using the elevated plus-maze and the shock-probe burying tests.

Table 1: A summary of the literature reviewing the effects of serotonergic manipulations on animal behaviour in the conflict test of anxiolysis. [+ = anti-conflict effect, 0 = no anti-conflict effect].

Manipulation	Reward	Results	Reference
5,7-DHT			
-ICV (200 µg) 3-5 days recovery	fluid	0	Commissaris et al., 1981
-ICV (450 µg) 14 days recovery	fluid	+	Soderpalm et al., 1991
-DR (3 µg) 21 days recovery	food	0	Thiebot et al., 1982
-DR (1 µg) 15 days recovery	food	+	Thiebot et al., 1983
-DR (1 µg) during training	food	0	Thiebot et al., 1984
-ventromedial tegmentum (2 µg) bilaterally during training	food	+	Tye et al., 1977
5,6-DHT			
-DR (10 µg)	food	+	Green et al., 1986
-ICV (100 µg)	food	+	Stein et al., 1975
p-CPA			
-IP (340 mg/kg) 3 days before testing	food	0	Blakely et al., 1973
-PO (60,100,200, 300 mg/kg) 17 hrs before testing	food	+ all doses	Geller et al, 1970
-IP (100 mg/kg) daily for 3 days	food	+	Green et al., 1983

Manipulation	Reward	Results	Reference
-CPA (con't)			
-PO (320 mg/kg) 12-20 hrs before testing	food	+	Hartmann et al., 1971
-IP (400 mg/kg or 200 mg/kg every two days	fluid	+	Kilts et al., 1982
-IP (400 mg/kg) SC (200 mg/kg)	fluid	+	Petersen et al., 1981
-PO (200 mg/kg) once every 6 days	food	+	Robichaud et al., 1969
-IP (100 mg/kg)	food	+	Shephard et al., 1982
-IP (316 mg/kg) 3 days before test	fluid	+	Stevens et al., 1969
-IP (300 mg/kg) daily for 3 days	fluid	+	Soderpalm et al., 1989.
-IP (100 mg/kg) daily for 3 days	food	+	Tye et al., 1979
Methylsergide			
-IP (1.25,5 mg/kg)	food	+	Cook et al., 1975
-IP (0.3,1,3,10, 30 mg/kg)	fluid	+ (at 1-30 mg/kg)	Graeff, 1974
-IM (pigeons) (0.1,0.3,1,3 mg/kg)	food	+ (at 0.3 mg/kg)	Graeff et al., 1970
-IP (1-10 mg/kg)	fluid	0 (- at 10 mg/kg 30 min prior to test)	Kilts et al., 1981
-IP (1,3,10,18 mg/kg)	fluid	0	Kilts et al., 1982

Manipulation	Reward	Results	Reference
Methylsergide (con't)			
-IP (0.3,1,3 mg/kg)	fluid	0	Petersen et al., 1981
-IP (10 mg/kg)	food	+	Stein et al., 1975
-IP (0.3,1,3,10 mg/kg)	food	+ (at 10 mg/kg)	Winter et al., 1972
Cyproheptadine			
-IP (1.7,3,5.6,10, 17 mg/kg)	fluid	+ (at 3-10 mg/kg)	Graeff, 1974
-IP (1,3,10,18 mg/kg)	fluid	0	Kilts et al., 1982
-IP (1,3,10 mg/kg)	fluid	0	Petersen et al., 1981
-IP (0.5-10 mg/kg)	fluid	+ (at 3 mg/kg)	Schoenfeld, 1976
Cinanserin			
-IP (15 mg/kg)	food	+	Cook et al, 1975
-IP (60 mg/kg)	food	+	Geller et al., 1974
-IP (10-100 mg/kg)	fluid	+ (at 56 mg/kg)	Kilts et al., 1981
-IP (10-100 mg/kg)	fluid	0 (at 56 mg/kg)	Kilts et al., 1982
-IP (10,30,60 mg/kg)	fluid	0	Petersen et al., 1981
-IP (3,10,20,25 mg/kg)	food	0	Winter, 1972

Manipulation	Reward	Results	Reference
Buspirone			
-SC (0.25,0.5,1 mg/kg)	fluid	+	Moser et al., 1990
-PO (2,5,10 mg/kg)	fluid	0	Gardner, 1986
8-OH-DPAT			
-DR (100 ng)	fluid	+	Higgens et al., 1987
-SC (12.5,25,50, 100,200 mg/kg)	fluid	0	Moser et al., 1990

EXPERIMENT 1

The purpose of the first experiment was to study the effects of p-CPA on plus-maze and shock-probe behaviours in the rat. Reductions of 5-HT levels in rats after p-CPA treatment occurs as a result of inactivation of the enzyme, tryptophan hydroxylase (Jequier, Lovenberg, & Sjoerdsma, 1967). The action of p-CPA inhibits this essential synthesis enzyme, preventing the formation of 5-HT in the body. Treatment with p-CPA has been shown to significantly reduce brain 5-HT concentrations 24 hours after a single injection (Koe & Weissman, 1966; Marsden & Curzon, 1977). Upon repeated injections of p-CPA, brain 5-HT levels have been depleted by as much as 90% in rats, without altering other neurochemical levels significantly (Koe & Weissman, 1966). Once p-CPA administration is terminated, the effects of p-CPA on 5-HT concentrations dissipate over time as new tryptophan hydroxylase is synthesized (Koe & Weissman, 1966). Since p-CPA is an effective means of depleting 5-HT, it was considered a useful tool in exploring the hypothesis that reduced 5-HT function leads to anxiolytic profiles in animal models of anxiety. It was hypothesized that treating rats with p-CPA would increase open arm activity in the plus-maze test and decrease burying in the shock-probe test.

Method

The plus-maze test: The elevated plus-maze apparatus

consisted of a wooden, four-arm, cross-shaped maze, each arm consisting of planks 50 cm long and 10 cm wide. Two opposite arms of the maze had three surrounding walls 50 cm high ("closed" arms) while the remaining two arms had no walls ("open" arms). A small 10x10 cm square area at the centre of the maze marked the junction of the four maze arms. All four maze arms were elevated 50 cm above the floor by a wooden support. The plus-maze was kept in a quiet testing room under dim illumination. Prior to testing, animals were handled for 30-60 seconds. Following this, rats were placed in the centre of the maze facing a closed arm. Animals were then allowed to freely explore the maze for five minutes while the experimenter observed the animal's behaviour from an angled mirror positioned to reflect the maze arms from above the maze. During the five minute trial, several measures were recorded, including the entries made into closed or open maze arms, and the time (in seconds) spent in the open or the closed arms. An entry was defined as all four paws entering the maze arm. Time spent in the junction of the maze arms was not recorded. From these measures, the percent time ([open/open+closed] x 100) in, and percent entries ([open/open+closed] x 100) into the open maze arms were calculated. An increase in the percentage of open arm activity (i.e., % time and % entries) indicates a reduction of "anxiety" in this test. Total numbers of entries into all maze arms was also measured as

an index of the overall activity level of the rats. The maze was cleaned after each test session.

The shock-probe burying test: The shock-probe chamber consisted of a Plexiglas box, 40 cm long, 30 cm wide, and 40 cm high, the floor of which was covered with 5 cm of absorbent bedding material (kitty litter). One end wall contained a hole, 2 cm above the bedding, through which a probe (6 cm long, 0.5 cm in diameter) wrapped with wire could be inserted. A video camera was focused on the chamber. The chamber could be observed via a monitor in an adjacent room, and behaviour in the chamber was recorded on standard VHS tapes using a VHS video recorder. Prior to testing, rats were placed in the chamber (without the probe) for daily 15 minute habituation sessions, over four consecutive days. On the fifth day (test day), the probe was inserted into the box and connected to a shock source set to deliver 2 mA of current through the probe. The video recorder was turned on, and the rats were individually placed in the box, away from the probe, with the current off. The observer then returned to the adjacent room to observe the animal. Once the rat made contact with the probe with forepaws or snout, the current was turned on, delivering a shock to the rat. The current was then left on for the remainder of the test session. The animal's behaviour was measured for 15 minutes following the first

shock. The behaviours measured during this 15 minute test session included the number of contact-induced shocks received from the probe, the rats' reactivity to shock (on a scale of 1-4, with 1=flinch, 2=flinch and step back from probe, 3=flinch, turn and walk to opposite end of chamber from probe, and 4=jump, vocalize, or attempt to escape the chamber. The highest reactivity score for each animal was used for analysis), the time spent burying (i.e., spraying bedding material toward the probe with forepaws), and the time spent immobile (no movement evident, whether standing, resting on hind-quarters, or lying on belly). In this test, a reduction in "anxiety" is indicated by a selective reduction in burying behaviour. After each test, the chamber was cleaned of feces and other debris, and bedding material was levelled to 5 cm. Animals failing to contact the probe after 5 minutes were discarded from data analysis.

p-CPA treatment: Preparations of p-CPA (DL-p-chlorophenylalanine methyl ester hydrochloride, obtained from Sigma Pharmaceuticals) were made fresh daily by dissolving p-CPA in sterile 0.9% saline to concentrations of 26 mg/ml. Injections of 130 mg/kg p-CPA were given IP in 5 ml/kg volumes. Control animals were injected IP with 5 ml/kg of 0.9% saline solution.

Subjects: Sixty experimentally naive male Spraque Dawley rats (obtained from Charles River breeding facilities, Quebec), weighing between 300-340 grams, were used in this

experiment. Rats were housed in shoe-box cages with wood shavings lining the floor, and were allowed ad lib. access to food and water. The colony room was maintained on a 12 hr light/dark cycle (lights on at 0700). Animals were maintained in the colony room for one week prior to beginning the experiment.

Procedure: Twenty-four hours prior to the first injection, rats were handled and tail-marked for identification. Injections of p-CPA were delivered to 30 animals over four consecutive days (130 mg/kg/day). This drug regimen has been shown to reduce brain 5-HT levels to approximately 10% of control levels (Koe & Weissman, 1966). The remaining 30 animals received daily injections of saline (5 ml/kg) for four consecutive days, and served as the control group. Prior to each daily injection of p-CPA or saline, half of the p-CPA-treated rats (n=15) and half of the saline-injected control rats (n=15) were placed in the shock-probe chamber for 15 minute habituation sessions, without the probe in place.

Behavioral testing occurred 24 hours after each rat's last injection. Rats given prior habituation sessions in the shock-probe apparatus (15 p-CPA-treated and 15 control animals) were tested in the shock-probe apparatus with the probe now inserted into one wall. The remaining animals (15 p-CPA-treated and 15 control animals) were tested on the plus-maze test. Separate groups of rats were used in each

behavioral test to ensure comparable 5-HT depletion levels on test day.

In an attempt to assess the time-course of the anxiolytic effects of p-CPA, rats originally tested in the shock-probe apparatus were tested the next day in the plus-maze, and rats originally tested in the plus-maze were re-tested in the same maze five days later.

Results & Discussion

In general, the results supported the idea that reduced 5-HT function produces anxiolytic-like effects in rats (see Figures 1 & 2). In the plus-maze, between-groups analysis of variance (ANOVA) revealed that p-CPA-treated rats spent a significantly greater percentage of time in the open arms of the maze compared to saline-injected rats ($F(1,28)=7.73$, $p<.01$). In addition, p-CPA-treated rats made a greater percentage of entries into the open maze arms compared to saline-injected control animals ($F(1,28)=14.75$, $p<.001$, see Figure 1). ANOVA was also performed on total entries into any arm to assess whether there were any differences in overall activity between the two groups of rats. While a significantly greater number of entries were made by p-CPA treated animals than by saline-injected animals ($F(1,28)=4.29$, $p<.05$), further analysis revealed that p-CPA-treated rats made significantly more open-arm entries than saline treated controls ($F(1,28)=14.10$, $p<.001$) but did not differ from controls in the number of closed arm entries

made ($F(1,28)=.05$, $p>.05$, see Table 2). These results confirm that p-CPA-treated animals showed a selective increase in open arm activity, rather than a generalized increase in overall activity.

Evaluation of rats' behaviour in the plus-maze test, two and six days after the last p-CPA injection, revealed attenuation of the "anxiolytic" effects of p-CPA. The effects of drug treatment were no longer significant 48 hours after the last p-CPA injection (% time, $F(1,28)=3.80$, $p>.05$; % entries, $F(1,28)=3.06$, $p>.05$), or six days after the last injection (% time, $F(1,28)=1.69$, $p>.05$: % entries, $F(1,28)=0.32$, $p>.05$, see Table 2). Research in our laboratory (Treit & Menard, 1991, unpublished observations) suggests this attenuation is not likely due to habituation of rats' open arm avoidance, as normal rats experiencing up to 18 repeated exposures to the plus-maze did not alter their usual avoidance of the open arms. Additionally, the group receiving plus-maze testing 48 hours after the last p-CPA injection had never been exposed to the plus-maze apparatus previously. It is more likely the attenuation of the antianxiety effects of p-CPA in the plus-maze occurred as a result of the recovery of 5-HT function with time (Chase et al., 1970; Koe & Weissman, 1966).

As predicted, p-CPA-treated rats showed a suppression in duration of burying compared to saline controls (see Figure 2). However, this difference just failed to reach

statistical significance ($F(1,28)=3.55$, $p>.07$). The two groups also did not differ significantly in the number of shocks received from the probe ($F(1,28)=0.406$, $p>.80$) or in their reactivity to shock ($F(1,28)=.057$, $p>.05$) suggesting p-CPA did not alter pain sensitivity, or the rats' ability to passively avoid the probe (see Table 2).

Animals treated with p-CPA did show increased levels of immobility compared to saline treated animals ($F(1,28)=6.68$, $p<.02$, see Table 2), suggesting the reduction in burying seen in p-CPA-treated rats was perhaps secondary to a more general motor impairment produced by p-CPA treatment. This increase in immobility was unexpected, as previous research has shown that p-CPA treatment produces hyperactivity in animals (Fibiger & Campbell, 1971; Jacobs, Trimbach, Eubanks, & Trulson, 1975; Marsden & Curzon, 1976; Marsden & Curzon, 1977), rather than hypoactivity. Additionally, the results of plus-maze testing revealed an elevation in total arm entries in p-CPA-treated rats compared with saline controls, suggesting p-CPA-treated animals were not motorically impaired in the plus-maze test. Thus, p-CPA-induced hyperactivity found by other researchers, and our own plus-maze results, suggest the increased immobility seen in p-CPA-treated rats during the shock-probe test was not due to motoric impairments.

"Immobility" can encompass several types of behaviours, such as sleeping, resting, standing still, or freezing

(where rats assume a rigid, upright posture accompanied by piloerection, after contact with an aversive or novel stimulus). The immobility displayed by p-CPA-treated rats in the shock-probe test was characteristic of that displayed by normal animals at the end of a habituation session in the shock-probe apparatus. During these periods, animals typically lay at one end of the chamber, either resting or sleeping. At no time did p-CPA-treated animals display more than a few seconds of freezing behaviour, and no gross ataxia was ever observed. Instead, the p-CPA-treated rats appeared to behave (e.g., sleeping, resting) in a manner consistent with anxiolysis.

In summary, the results of Experiment 1 suggest that a reduction of 5-HT function can produce anxiolytic behavioral effects. The plus-maze data revealed clear anxiolytic effects of p-CPA treatment, which dissipated with time after the last p-CPA injection. The shock-probe test produced a non-significant decrease in burying, which was accompanied by a significant increase in immobility. Although not as impressive as the plus-maze results, the shock-probe data were consistent with an anxiolytic effect. More importantly, the overall pattern of results from both tests (i.e., an increase in open arm activity and a decrease in burying behaviour) provide support for the hypothesis that a reduction in 5-HT function can produce a reduction in experimental "anxiety".

Figure 1: Mean (+/- SEM) percent time (open bars) in, and percent entries (hatched bars) into the of open arms of the plus-maze test by saline- and p-CPA-treated rats in Experiment 1

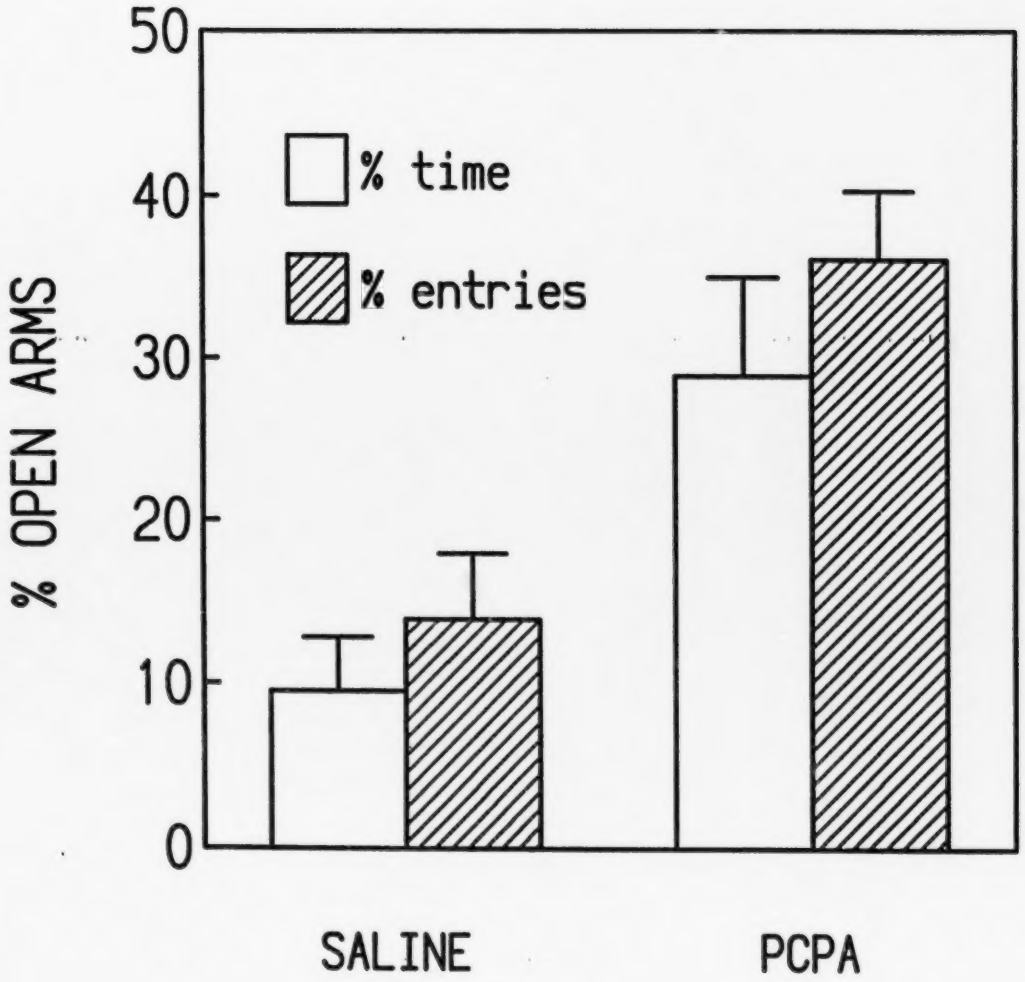

50
40
30
20
10
0
% OPEN ARMS
% time
% entries
SALINE
PCPA

Figure 2: Mean (+/- SEM) duration of time spent (in seconds) burying the probe in saline- and p-CPA-treated rats in Experiment 1.

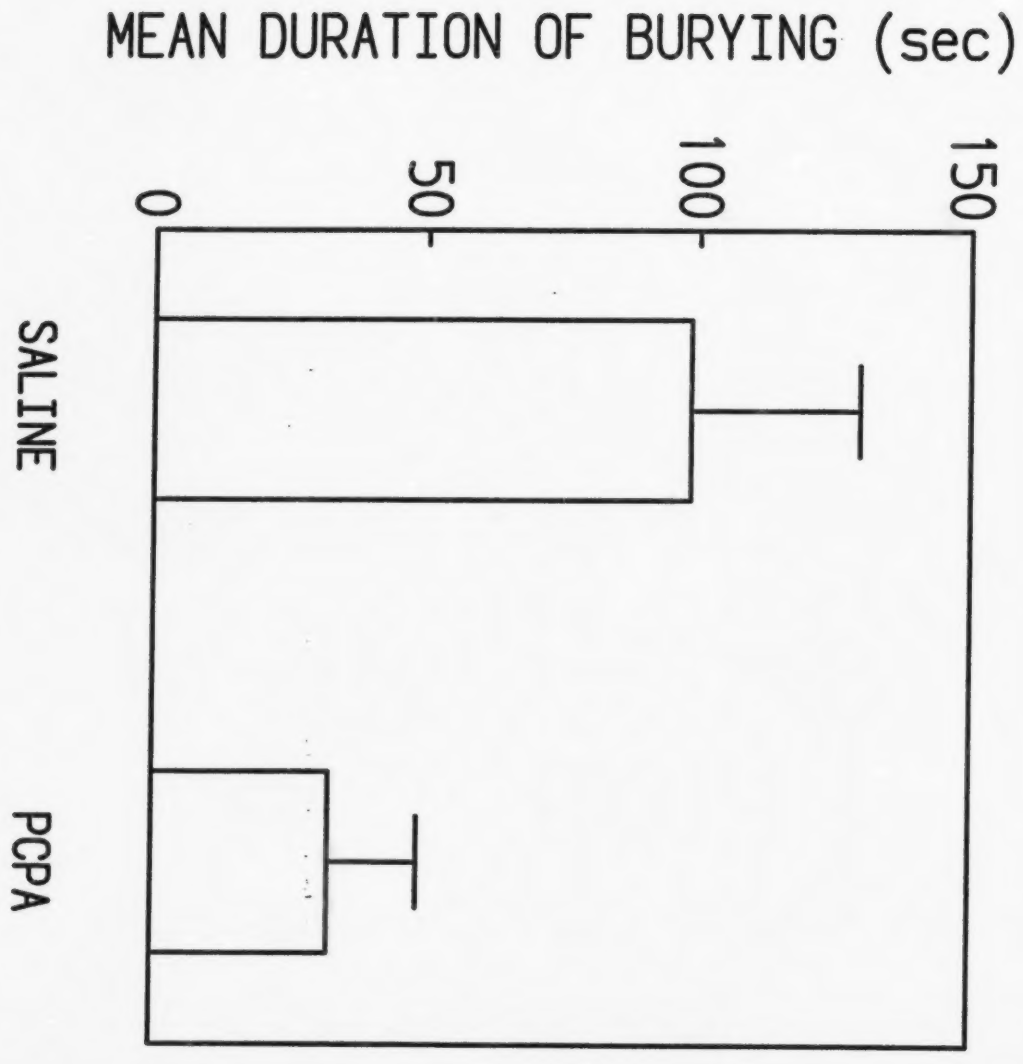

MEAN DURATION OF BURYING (sec)
0
50
100
150
SALINE
PCPA

Table 2: Mean (+/- SEM) values for total-arm entries, open-arm entries, closed-arm entries, immobility, probe contacts, and shock reactivity for rats in Experiment 1.

Measure	Group	Mean	SEM
Total # entries into maze arms	pCPA	10.3	1.07
	SAL	7.5	0.72
Entries into open arms	pCPA	3.9	0.58
	SAL	1.3	0.37
Entries into closed arms	pCPA	6.47	0.63
	SAL	6.27	0.03
Immobility	PCPA	306.7	55.5
	SAL	127	41.9
Probe contacts	pCPA	1.66	0.19
	SAL	1.73	0.25
Shock reactivity	pCPA	2.27	0.15
	SAL	2.33	0.16
<u>48 hrs post-pCPA</u>			
% entries	pCPA	8.7	3.41
	SAL	8.3	1.16
% time	pCPA	16.0	8.13
	SAL	6.0	2.03
<u>5 days post-pCPA</u>			
% entries	pCPA	2.9	1.92
	SAL	3.9	2.58
% time	pCPA	1.0	0.72
	SAL	1.0	0.33

EXPERIMENT 2

The purpose of the second experiment was to explore the effects of electrolytic lesions of raphe nuclei on rat behaviour in the plus-maze and shock-probe tests. Unlike p-CPA, electrolytic raphe lesions deplete brain 5-HT but do not dramatically alter peripheral 5-HT levels (Jacobs, Wise & Taylor, 1974). Thus, electrolytic lesions of the raphe allow investigation of the behavioral effects of CNS 5-HT depletion. Furthermore, the anatomical specificity of the lesion technique allows exploration of the relative contribution of different populations of 5-HT neurons in mediating animal "anxiety".

In this experiment, the effects of combined electrolytic lesions of the dorsal raphe (DR) and median raphe (MR) on animal behaviour in the plus-maze and shock-probe tests were investigated. Previous experiments have shown that raphe lesions can produce anti-conflict effects in the conflict paradigm (see introduction). In addition, the DR and MR both provide 5-HT afferents to several forebrain structures , including limbic areas such as the hippocampus, the septum and the amygdala (Azmitia & Segal, 1978; Consolazione & Cuello, 1982). Since many of these limbic areas have previously been implicated in experimental anxiety (Clarke & File, 1982; Gray, 1982; Kostowski et al., 1989; Treit & Pesold, 1990), it is possible that 5-HT connections between these limbic areas and the raphe might

mediate anxiety in animals.

Given that the DR and MR are the important 5-HT-containing structures mediating "anxiety", it was expected that combined electrolytic lesions of the DR and MR would produce anxiolytic behavioral profiles in both the plus-maze and shock-probe tests.

Method

Surgery: Prior to undergoing surgery, 29 experimentally naive, male Sprague Dawley rats (250-340 grams) were anaesthetized using a combination of sodium pentobarbital (20 mg/kg IP) and ketamine hydrochloride (60 mg/kg IM). When necessary, supplemental doses of chloral hydrate (approximately 100 mg/kg IP maximum dose) were administered to achieve full anaesthesia.

Once anaesthetized, animals were secured in a stereotaxic apparatus (Kopf instruments). An incision was made in order to expose the skull. Flat skull coordinates for electrode placements were derived from pilot studies using Paxinos and Watson's (1986) stereotaxic atlas of the rat brain, with bregma as a skull reference point. As the DR and MR are midline structures, electrode placements were made at an angle, bilaterally, in order to avoid the dural venous sinus. Coordinates for the DR were 7.7 mm posterior, 2.1 mm lateral, and 6.25 mm ventral from dura, with the electrode angled toward the midline 20 degrees from vertical. Lesions of the DR were produced using 0.8 mA

anodal current delivered through an electrode insulated to within .5 mm of the tip, for 10 seconds on each side. Coordinates for the MR were 7.7 mm posterior, 1.4 mm lateral, and 8.4 mm ventral from dura, with the electrode angled at 10 degrees from vertical. Lesions of the MR were produced by passing 0.8 mA through the electrode for 4 seconds on each side. In sham-lesioned animals, the electrode was lowered into the DR and MR, but no current was delivered. After surgery, the incision was sutured and animals were allowed to recover under a warm lamp until they were semi-conscious. Animals were then returned to their home cages in the colony room. Periodically, animals were observed until they had fully recovered from surgery. In this experiment, 19 experimental rats received electrolytic lesions and 10 control rats received sham lesions.

Procedure: The behavioral testing procedures were similar to those in Experiment 1, except that all animals were tested in both the plus-maze and the shock-probe tests. Seven days post-surgery, all animals were handled by the Experimenter. Additional handling occurred daily over the next two days until Day 10 post-surgery. At this time, all rats were tested in the plus-maze, using the same procedure described in Experiment 1. Immediately following plus-maze testing, all animals began the first of four habituation sessions in the shock-probe chamber (without the probe in place). After habituation, fourteen days post-surgery, all

animals were tested in the shock-probe apparatus. Again, the same procedure described in Experiment 1 was used for shock-probe testing.

Histological analysis: Following behavioral testing, animals were given an overdose of chloral hydrate prior to perfusion intracardially with 0.9% saline, followed by 10% formaldehyde fixative (formalin). Following fixation, brains were extracted from the skull and allowed to sit in formalin for at least one week prior to dissection. For dissection, brain segments were frozen to mounting platforms in dry ice, then were transferred to a cryostat, where serial coronal sections (40 microns thick) were taken. Every third section through the area of the lesion was placed on a gelatin-coated slide and allowed to dry overnight. The following day, these slices were stained with thionin cell stain, and preserved with permount for later lesion analysis.

According to the Paxinos & Watson stereotaxic atlas, the region of the DR extends from -7.1 to -9.3 posterior from bregma, below the cerebral aqueduct, above the decussation of the superior cerebellar peduncle, and between the dorsal tegmental bundles. The MR extends from -7.3 to -8.3 posterior from bregma, below the decussation of the superior cerebellar peduncle, above the reticulopontine nuclei of the pons and pontine nuclei, and bordered by the oral pontine reticular nuclei and the para-median raphe.

Lesions were considered acceptable provided they fulfilled the following criteria; complete or near-complete (aprox. 80%) lesions of the DR and MR, with restricted (generally localized within 0.5 mm of the intended site) or no extra-raphe damage. 'Blind' analysis of lesion placements showed only 8 subjects sustained acceptable combined lesions of the DR and MR as outlined (see Figure 5). Only data from these animals and from sham-lesioned animals were included in the subsequent statistical analysis of behavioral measures.

Results & Discussion

The results of Experiment 2 were generally consistent with those of Experiment 1. When tested in the plus-maze, rats that had sustained lesions of the DR and MR displayed a clear anxiolytic profile compared to sham-lesioned controls (see Figure 3). Statistical analysis using a between-groups, unweighted means ANOVA revealed a significant increase in the percent time lesioned animals spent in open arms ($F(1,16)=24.21$, $p<.001$) and in the percent entries into open arms ($F(1,16)=32.66$, $p<.001$) compared to the sham-lesioned control group. The total arm entries made by lesioned animals was also significantly greater than sham animals ($F(1,16)=5.86$, $p<.05$), but as in Experiment 1, further analysis revealed that this was due to a selective increase, compared to controls, in open arm entries ($F(1,16)=22.72$, $p<.001$), rather than a change in closed arm entries ($F(1,16)=1.13$, $p<.05$, see Table 3). This selective

increase in open arm activity in raphe-lesioned animals was clearly indicative of an anxiolytic profile.

The results from the shock-probe test were somewhat similar to those of Experiment 1. Raphe-lesioned rats showed a substantial but non-significant decrease in burying compared to the sham group ($F(1,16)=1.38$, $p>.05$; see Figure 4). Unlike Experiment 1, however, lesioned rats did not show a significant change in immobility compared to sham-lesioned rats ($F(1,16)=.23$, $p>.05$). There were no significant differences in the number of shocks received by the lesioned and sham animals ($F(1,16)=.009$, $p>.05$), nor were there any differences between lesioned or sham groups in their reactivity to shock ($F(1,16)=0.00$, $p>.05$, see Table 3). Thus, there was no evidence suggesting altered pain sensitivity or passive avoidance deficits in the lesioned animals.

Only 8 of the original 19 experimentally lesioned rats sustained combined lesions of the DR and MR within the inclusion criteria discussed earlier. Of these 8 lesions, no damage was evident in the substantia nigra (a major DA-containing region) or the locus ceruleus (a major NE-containing region). However, slight extra-raphe damage was observed in some areas lying proximal to the dorsal and median raphe (i.e., oculomotor nuclei, caudal linear raphe, superior cerebellar peduncle, peri-aqueductal gray, dorsal tegmental bundle, tegmental nuclei, interpeduncular nuclei,

medial lemniscus, certain pontine nuclei, and the paramedian raphe). Despite the number of extra-raphe structures sustaining some damage, the actual area of extra-raphe damage in any lesioned animal included in the lesion group was within the outlined criteria for inclusion. It is interesting to note that despite some extra-raphe damage, lesioned animals showed a behavioral profile very similar to animals treated with p-CPA, suggesting the effects of these electrolytic lesions were due to reductions in 5-HT, and not due to a secondary effect of some extra-raphe damage.

Overall, it appears disruption of 5-HT via electrolytic lesions of the tegmental raphe nuclei (the DR and MR) produces a robust increase in open arm activity in the plus-maze test and a non-significant decrease in burying in the shock-probe test. This pattern of results is quite consistent with results of Experiment 1, where 5-HT was depleted using p-CPA.

Figure 3: Mean (+/- SEM) percent time (open bars) in, and percent entries (hatched bars) into the of open arms of the plus-maze test by sham- and combined DR and MR-lesioned rats in Experiment 2.

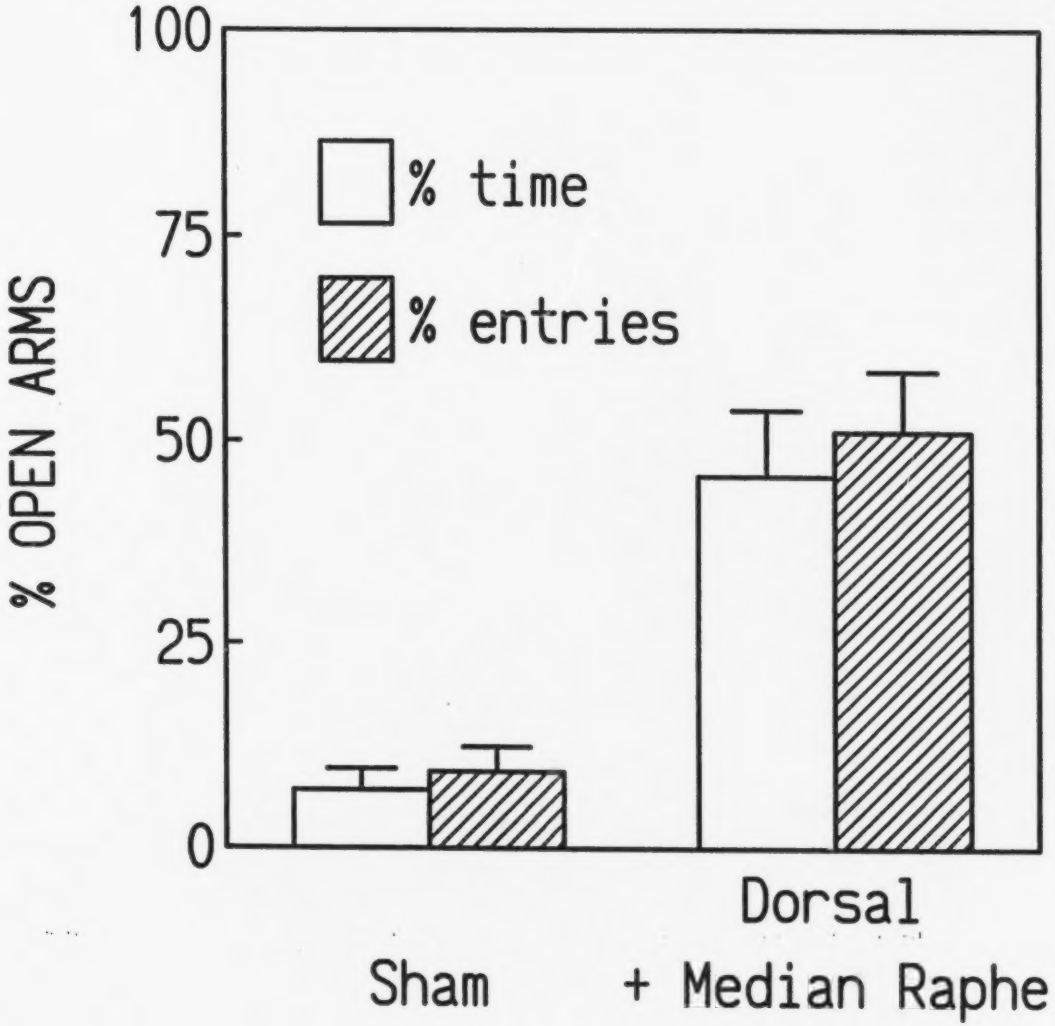

100
75
50
25
0
% OPEN ARMS
% time
% entries
Dorsal
Sham
+ Median Raphe

Figure 4: Mean (+/- SEM) duration of time spent (in seconds) burying the probe in sham- and combined DR and MR-lesioned rats in Experiment 2.

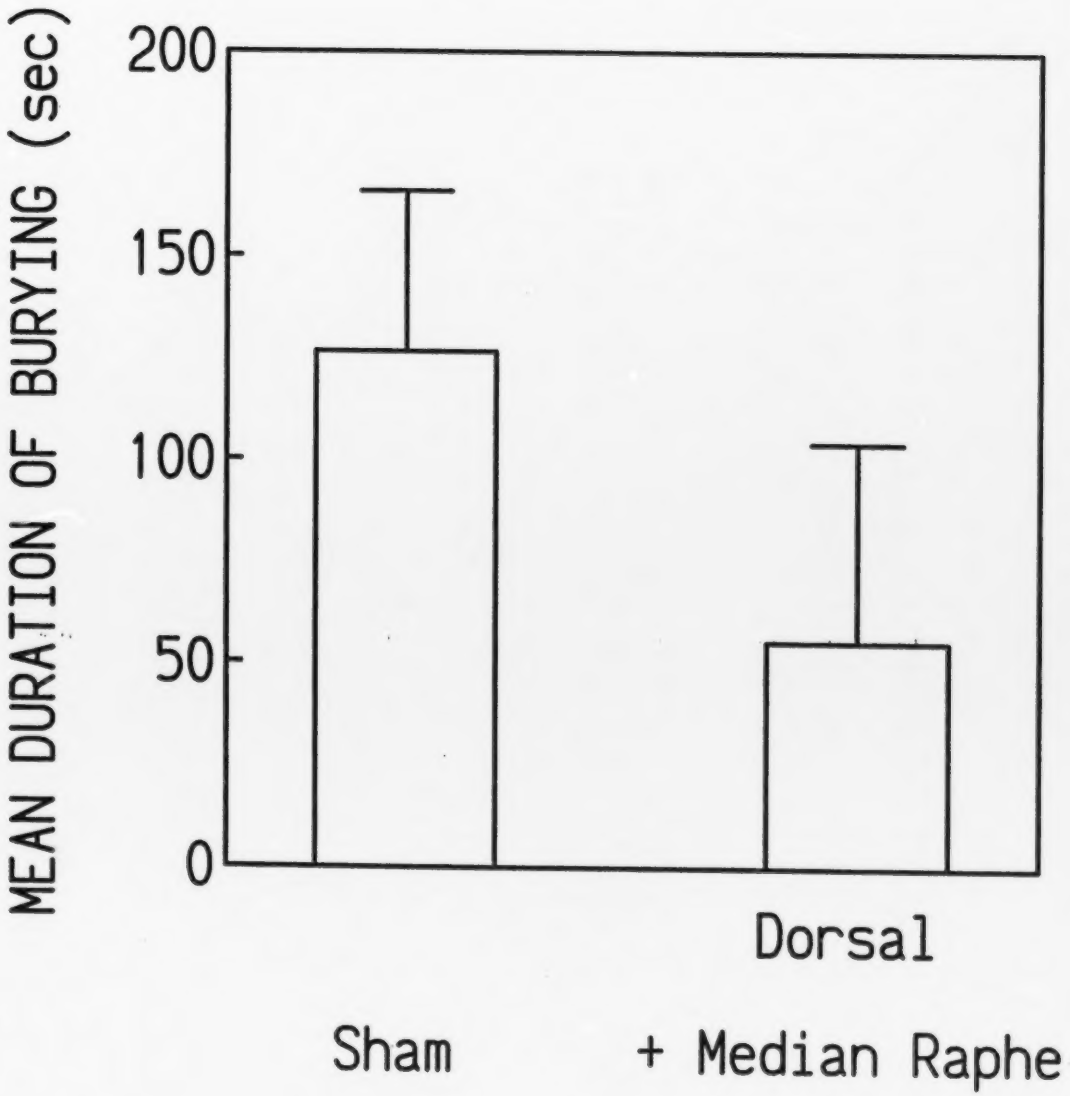
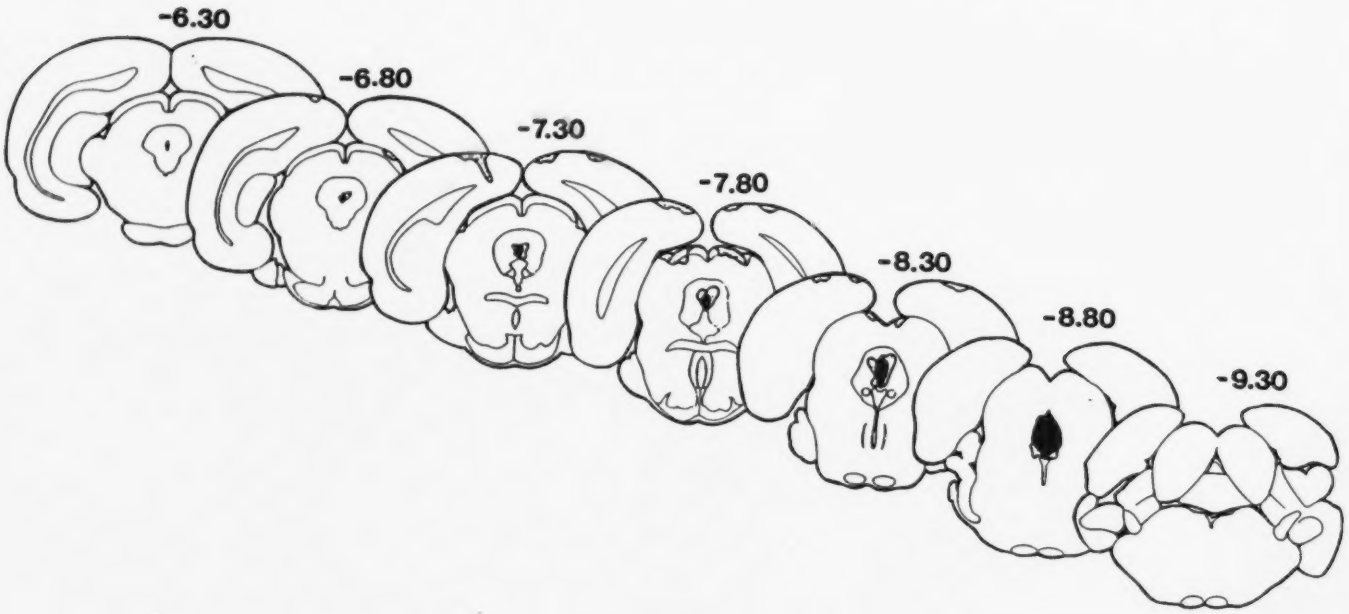

MEAN DURATION OF BURYING (sec)
200
150
100
50
0
Dorsal
Sham
+ Median Raphe

Figure 5: Histological representation of sham lesions (A) and combined raphe lesions (B). Light shading represents the maximum extent of the lesions, while dark shading represents a typical lesion. Numbers above each section indicate distance (in mm) from bregma.

A



B

Table 3: Mean (+/- SEM) values for total-arm entries, open-arm entries, closed-arm entries, immobility, probe contacts, and shock reactivity for rats in Experiment 2.

Measure	Group	Mean	SEM
Total # entries into maze arms	DMR	15.6	2.03
	SHAM	10.3	1.13
Entries into open arms	DMR	8.1	1.63
	SHAM	1	0.33
Entries into closed arms	DMR	7.5	1.28
	SHAM	9.3	1.12
Immobility	DMR	77.3	74.3
	SHAM	41.4	30.1
# Shocks taken	DMR	2.25	0.28
	SHAM	2.20	0.42
Shock reactivity	DMR	2.50	0.38
	SHAM	2.50	0.31

EXPERIMENT 3

The purpose of the third experiment was to compare electrolytic lesions of the DR with lesions of the MR in producing anxiolytic profiles in the plus-maze and the shock-probe tests. Anatomical evidence shows that there is considerable overlap in connections between the DR and MR nuclei and the limbic system (Azmitia & Segal, 1978). Furthermore, selective lesions of either the DR or MR have been shown to reduce 5-HT levels to a similar extent in several forebrain structures (Jacobs, Wise, & Taylor, 1974). Despite the similarities in DR and MR connections, however, there is some evidence suggesting the DR and MR may be differentially involved in different types of behaviours. For example, electrolytic lesions of the MR produce hyperactivity in animals, whereas lesions of the DR do not (Jacobs et al., 1974; Srebro & Lorens, 1975).

To date, no researchers have compared the effects of selective electrolytic lesions of the MR or DR, on animal "anxiety". Instead, most researchers have focused on the contribution of the DR in mediating anxiolysis in animals. For example, a number of studies (reviewed in the introduction) have shown that lesions and neuropharmacological manipulations that reduce 5-HT function in the DR also produce anxiolysis in the conflict test (Green & Hodges, 1986; Thiebot et el., 1982; Thiebot et al, 1983). In addition, the dorsal raphe (DR) has been linked

to fear-like behaviour in tree-shrews (Walletschek & Raab, 1982), rats (Kiser, Brown, Sanghera, & German, 1980), and non-human primates (Higley, Suomi, & Linnoila, 1990). Few neuropharmacological studies have compared the contribution of the DR and MR in mediating *drug* effects in animal models of anxiety (e.g., Carli, Prontera, & Samanin, 1989), and no research has investigated the effects of MR *lesions* on anxiety in animals. The purpose of Experiment 3, therefore, was to directly compare the effects of electrolytic DR and MR lesions on experimental anxiety in the plus-maze and shock-probe tests.

Method

In Experiment 3, 50 experimentally naive, male Sprague Dawley rats, weighing between 280-380 grams, underwent surgical procedures similar to those described in Experiment 2, except that bilateral lesions were now restricted to either the DR or the MR. The coordinates for the DR were 7.6 mm posterior, 2.1 mm lateral, 6.2 mm ventral from dura, with the electrode angled toward the midline 20 degrees from vertical, while coordinates for the MR were 7.7 mm posterior, 1.1 mm lateral, and 8.4 mm ventral from dura, with the electrode angled 10 degrees from vertical. In this experiment, 16 rats received lesions in the region of the DR, 7 rats received sham DR lesions, 18 rats received lesions in the region of the MR, and 9 rats received sham MR lesions. All other aspects of the surgical procedures were

the same as those described in Experiment 2. Prior to behavioral testing, animals were handled from Day 7 to Day 9 post-surgery, tested on the plus-maze on Day 10, followed immediately by the first of four habituation sessions in the shock-probe chamber. On Day 14, rats were tested in the shock-probe apparatus. Following behavioral testing, histological analysis was performed as described in Experiment 2, except that luxol fast blue/neutral red counterstaining was used to stain brain tissue. The criteria for acceptable lesions of either the DR or MR followed the same guidelines as described in Experiment 2 (complete or 80% lesion of the targeted area, with minimal extra-raphe damage) except that lesions targeting the DR could not include any MR damage, and lesions targeting the MR could not include DR damage. Blind histological analysis confirmed 7 acceptable DR lesions and 11 acceptable MR lesions based on these criteria (see Figure 8). Only the behavioral data from animals with acceptable DR or MR lesions, and from the sham-lesioned animals, were included in the subsequent statistical analysis. Additionally, the data from the two sham groups (DR shams and MR shams) were combined in cases where preliminary t-tests comparing their behavioral data revealed no significant differences between groups.

Results & Discussion

The results of Experiment 3 were not consistent with

the results of the first two experiments, although there was some evidence of an anxiolytic effect in the plus-maze (see Figures 6 and 7). In the plus-maze, an unweighted means ANOVA revealed a significant difference between groups in the percentage of time spent in open arms ($F(2,31)=3.37$, $p<.05$). Newman-Keuls post-hoc analysis further revealed that the DR-lesioned animals spent a greater proportion of their time in the open arms than sham-lesioned animals ($p<.05$, see Table 4), while MR-lesioned rats were not significantly different from controls on any plus-maze measure. For the percent entries measure, a preliminary t-test revealed a significant difference between the two sham groups, and the sham data were therefore not combined. Between-group differences for percent entries into open arms just failed to reach significance ($F(3,30)=2.49$, $p>.07$), although the general pattern of results was the same as for percent time (see Figure 6). There were no group differences in the total number of arm entries made ($F(2,31)=1.86$, $p>.05$). Thus, the effects of either DR or MR lesions in the plus-maze were not as impressive as the effects of combined (DR and MR) lesions found in Experiment 2, although the DR-lesioned animals did show some evidence of "anxiolysis" in the plus-maze test.

The results of the shock-probe test provide little evidence that electrolytic lesions of the DR or the MR produce anxiolysis in rats. There were no significant

differences between any of the groups in the duration of burying behaviour ($F(2,29)=2.28$, $p>.05$, see Figure 7). Despite this lack of significance, inspection of the group means shows that the DR-lesioned animals buried as long as the sham-lesioned group, and that MR-lesioned animals actually buried nearly three times longer than the former two groups. This effect suggests, if anything, that MR lesions have an anxiogenic (anxiety-producing) effect in the shock-probe test, rather than an anxiolytic effect. Nevertheless, it is important to note that the levels of burying displayed by control animals in this experiment were well below the levels displayed by control animals in Experiments 1 or 2 (see Figures 2 and 4). For this reason, and because there was no statistically significant difference between groups, it may not be prudent to place much emphasis on the pattern of group means seen in this experiment.

Despite the lack of significant differences in burying behaviour between the two lesion groups and the sham group, lesions did produce some significant changes in behaviour during shock-probe testing. Statistical analysis revealed that there was a significant group difference in animals' reactivity to shock during shock-probe testing ($F(2,29)=4.62$, $p<.02$). Although Newman-Keuls analysis failed to reveal a significant difference between the lesioned groups and the control group, the DR-lesioned

animals appeared to show lower shock reactivity than the MR or sham-lesioned animals (see Table 4). Since the amount of burying exhibited by DR-lesioned animals was not significantly different compared to the other two groups, it seems unlikely the reduced reactivity was a result of analgesia, as analgesia would likely reduce the amount of burying displayed by these animals.

Analysis also revealed significant group differences in the number of probe contacts made during shock-probe testing ($F(2,29)=3.88$, $p<.05$). Subsequent Newman-Keuls analysis revealed that animals with lesions of the MR made significantly more probe contacts (i.e., received more shocks) than either the DR-lesioned or sham-lesioned animals ($p<.05$). Some experiments have shown an increase in probe contacts accompanies decreased burying in the shock-probe test after anxiolytic drug administration (Treit, 1990; Treit & Fundytus, 1988). However, in this experiment, MR lesions did not decrease burying behaviour in the shock-probe test, and produced no evidence of anxiolysis in the plus-maze test. For these reasons, it is unlikely the increased number of probe contacts reflects anxiolytic effects of MR lesions. It is also unlikely the increased number of probe contacts was due to altered pain sensitivity, as MR-lesioned rats did not differ from sham-lesioned animals in their shock reactivity (see Table 4), and appeared to bury more than sham animals (see Figure 7).

Finally, it is unlikely MR-lesioned animals failed to learn an association between the shock and the probe. Failure to associate shock with the probe should have interfered with the burying response in MR-lesioned animals, but this did not occur. MR-lesioned animals buried the probe more than sham-lesioned animals.

The histological analysis for this experiment warrants discussion at this point. While lesions of the DR and the MR were within the criteria outlined earlier in this experiment (80% or more target damage, limited extra-raphe damage), there were very few lesions which consisted of full target damage, with no extra-raphe damage. Instead, there appeared to be a number of DR lesions only 80% complete, accompanied by some degree of extra-raphe damage. Lesions of the MR, while often complete, were also accompanied by extra-raphe damage. While the locus ceruleus and substantia nigra were spared in all accepted lesions, the damage to other proximal regions (detailed in Experiment 2) was more extensive than in Experiment 2. Additionally, animals with acceptable DR lesions appeared to show sparing of the anterior portion of the DR region. This anterior portion of the DR appears to be essential in 5-HT transmission to the forebrain, as sparing of these cell bodies (and the axons of passage from more posterior 5-HT cells), appears to limit the degree of 5-HT depletion in the forebrain significantly (Lorens & Guldberg, 1974). In comparison with lesions

obtained in Experiment 2, the lesions in this experiment were more variable, and in the DR, less complete. These anatomical factors may have contributed to the variability in the behavioral data seen in this experiment. Despite this problem, however, the plus-maze data from this experiment suggest that of the DR and MR regions, the DR may be more important in mediating animal anxiety.

Figure 6: Mean (+/- SEM) percent time (open bars) in, and percent entries (hatched bars) into the of open arms of the plus-maze test by sham-, DR-, and MR-lesioned rats in Experiment 3.

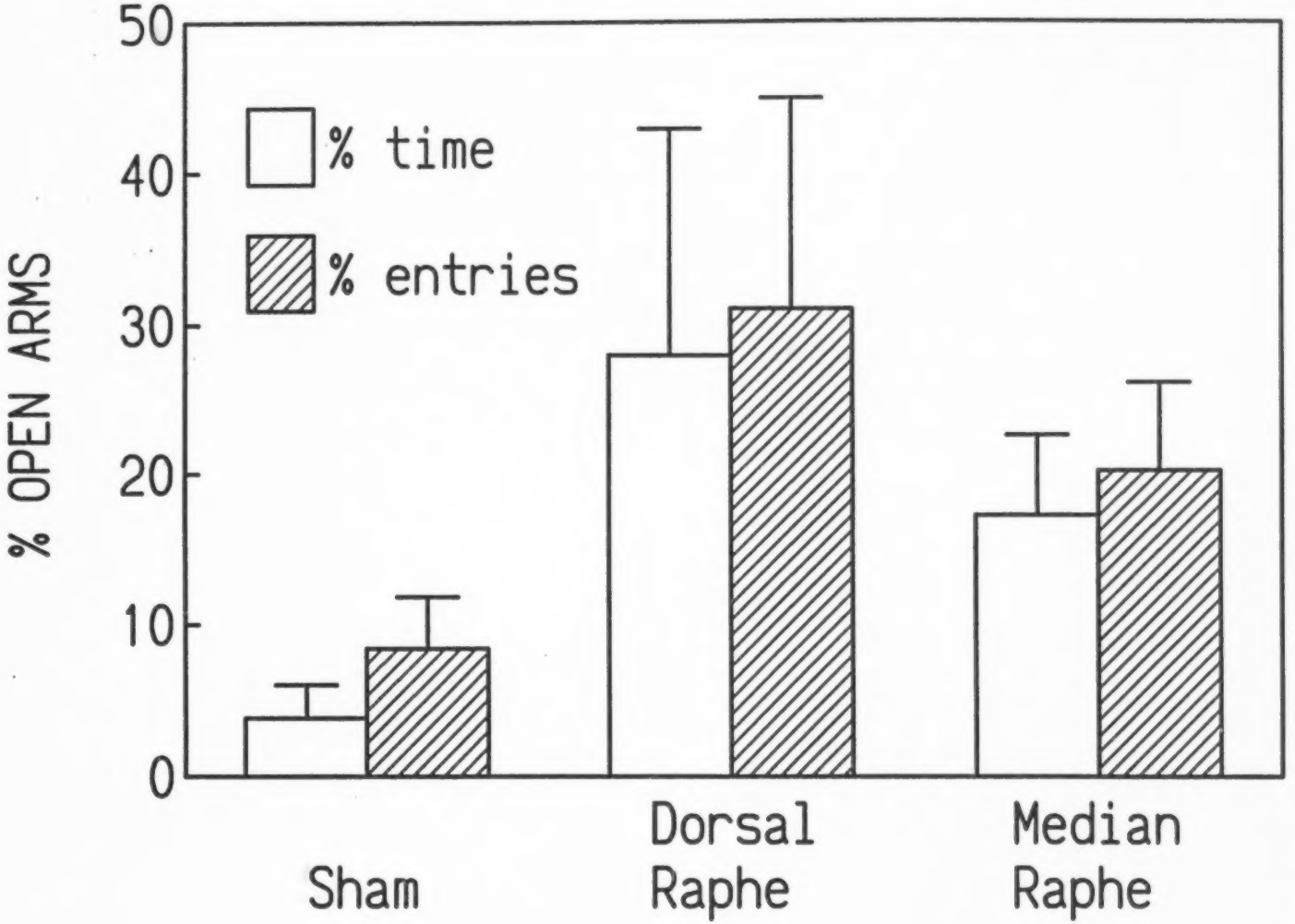

50
40
30
20
10
0
% OPEN ARMS
% time
% entries
Sham
Dorsal Raphe
Median Raphe

Figure 7: Mean (+/- SEM) duration of time spent (in seconds) burying the probe in sham-, DR-, and MR-lesioned rats in Experiment 3.

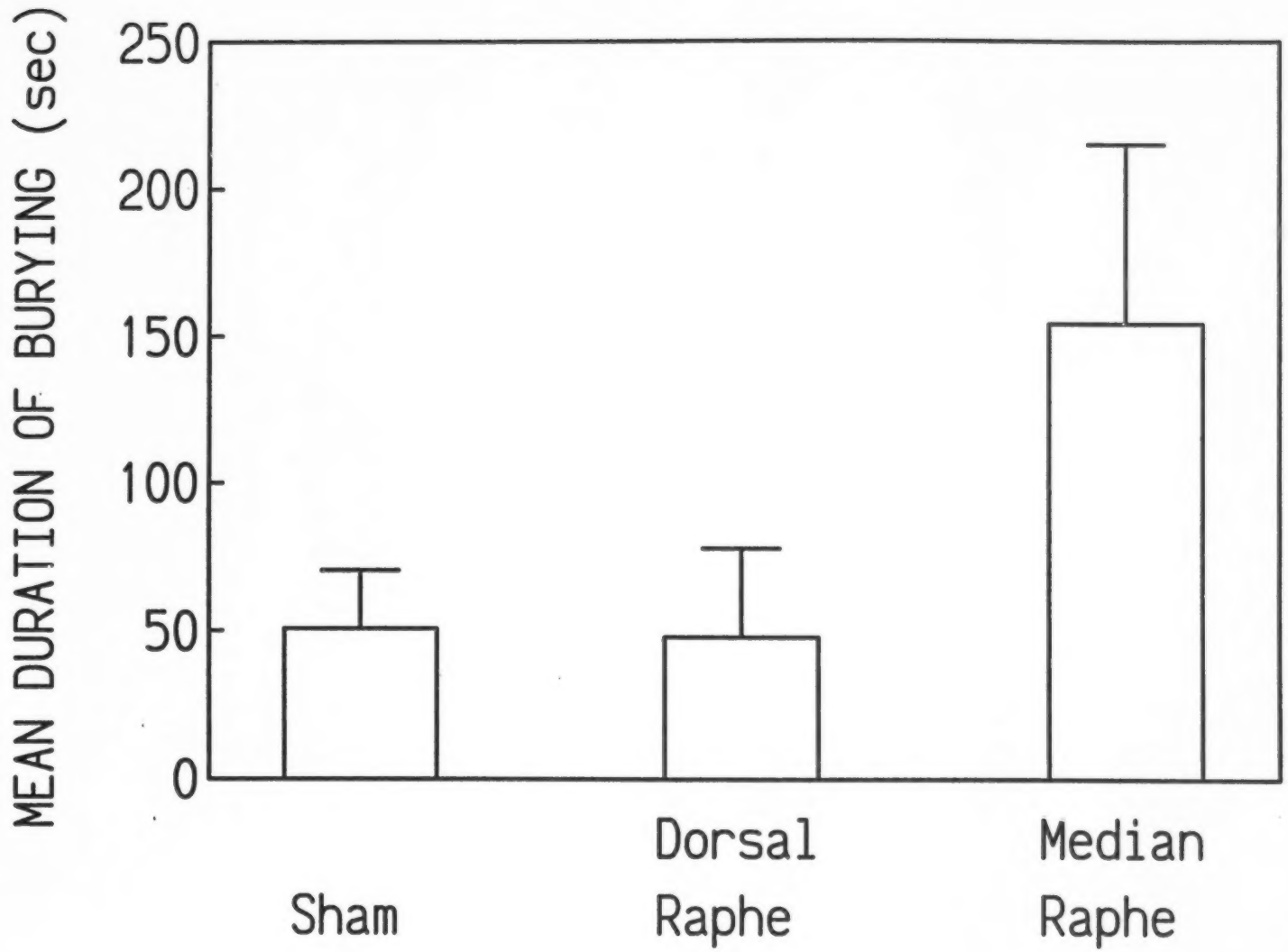

MEAN DURATION OF BURYING (sec)
250
200
150
100
50
0
Sham
Dorsal Raphe
Median Raphe

Figure 8: Histological representation of sham lesions (A), DR lesions (B), and MR lesions (C). Light shading represents the maximum extent of the lesions, while dark shading represents a typical lesion. Numbers above each section indicate distance (in mm) from bregma.

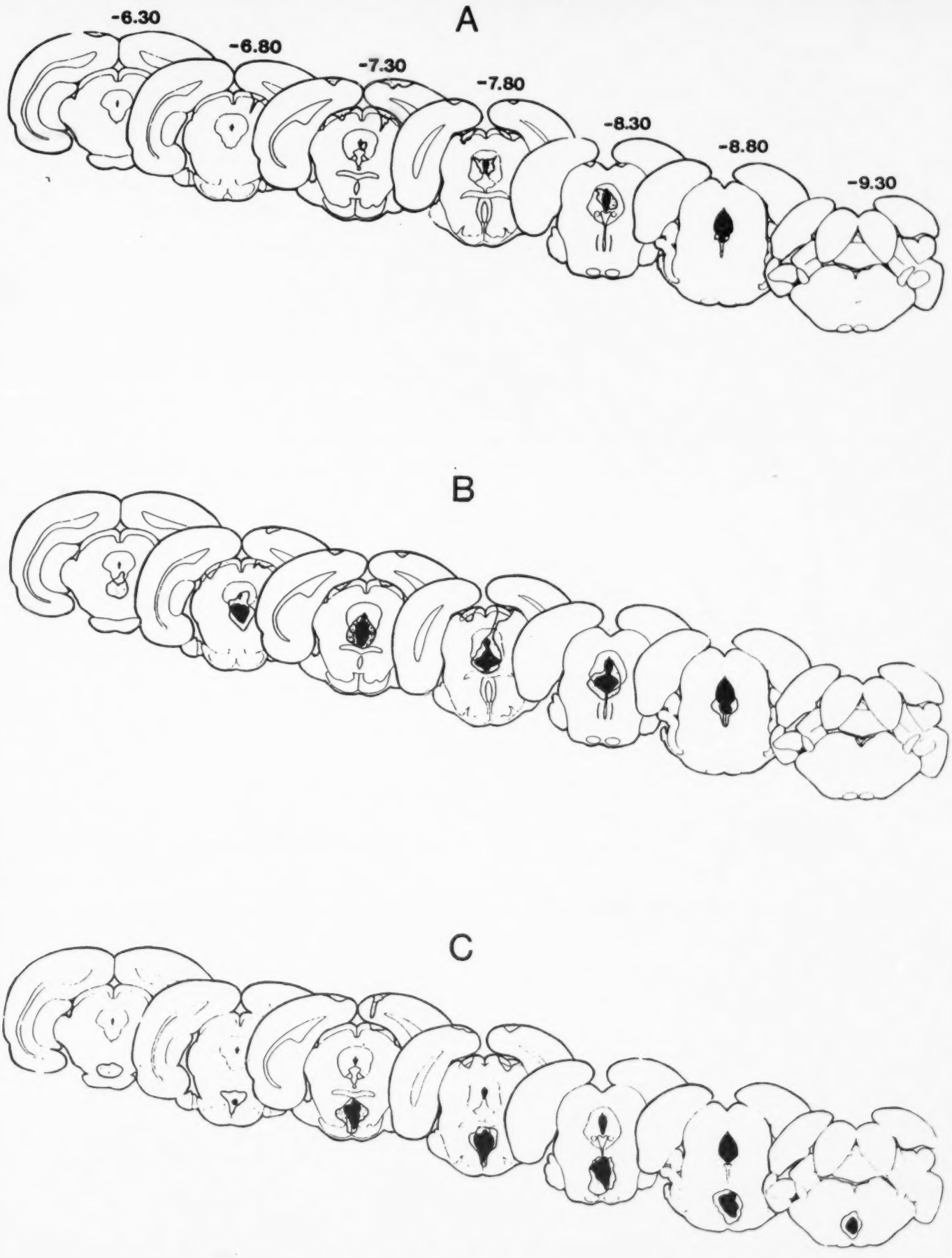

A
-6.30
-6.80
-7.30
-7.80
-8.30
-8.80
-9.30
B
C

Table 4: Mean (+/- SEM) values for total-arm entries, open-arm entries, closed-arm entries, immobility, probe contacts, shock reactivity, post-hoc comparisons, and Newman-Keuls Q values for rats in Experiment 3.

Measure	Group	Mean	SEM
Total # entries into maze arms	MR	11.0	1.86
	DR	7.70	1.64
	SHAM	10.2	0.97
Immobility	MR	178.5	157.3
	DR	51.7	20.5
	SHAM	105.4	25.9
# Shocks taken	MR	2.20	0.24
	DR	1.57	0.29
	SHAM	1.33	0.16
Shock reactivity	MR	2.50	0.31
	DR	1.57	0.20
	SHAM	2.10	0.12

Measure	Comparison	Newman-Keuls Q	DF	p
% time in open arms	DR > SHAM	Q=24.6	(31)	<.05
# Shocks	MR > SHAM	Q=.87	(29)	<.05

EXPERIMENT 4

The purpose of the fourth experiment was to further explore the effect of electrolytic lesions of the DR on anxiolysis in rats. The results from Experiment 3 suggested that the DR might be more important than the MR in mediating animal "anxiety" since open-arm activity was significantly enhanced by DR lesions, but not by MR lesions. Additionally, previous research into the role of 5-HT systems in mediating "conflict" effects in rats has emphasized the importance of the DR (Green et al., 1986; Higgens et al., 1987).

A second issue addressed in experiment 4 was the failure to find clear anxiolytic results in the shock-probe test after 5-HT reduction in Experiments 1, 2, and 3. While 5-HT disruption did produce anxiolytic-like reductions in burying in some of these experiments, the reductions were not statistically significant, and were often accompanied by other behavioral effects (i.e., increased immobility). In contrast, 5-HT disruption consistently produced significant (anxiolytic) increases in open arm activity in Experiments 1, 2, and 3. Although this difference in the reliability of "anxiolytic" effects might reflect a differential sensitivity of the two tests to 5-HT manipulations, the failure to see clear 5-HT-mediated anxiolysis in the shock-probe test might also reflect unintended procedural variation during the conduct of the shock-probe test, which

might have rendered the test insensitive to any anxiolytic manipulation.

In order to demonstrate the procedures used in these experiments were sufficient to detect anxiolytic decreases in burying in the shock-probe test, a second "positive" control group, receiving electrolytic lesions of the posterior septum (PS), was included. Electrolytic lesions of the PS have produced robust reductions in probe burying behaviour in our laboratory (Treit & Pesold, 1990; Pesold & Treit, 1992), and in another laboratory (Gray, Terlecki, Treit, & Pinel, 1981), as well as a reliable increase in open-arm activity in the plus-maze (Treit & Pesold, 1990). If the PS-lesioned animals do show a significant decrease in burying, but DR-lesioned animals do not, it would suggest that procedural error was not responsible for the lack of significant effects in the shock-probe test seen after 5-HT depletion in previous experiments.

Method

Thirty-four experimentally naive, male Sprague Dawley rats (240-400 grams) were used in this experiment. Electrolytic lesions were performed using the same general procedures used in Experiments 2 and 3. Coordinates for the DR were 7.7 mm posterior, 2.1 mm lateral, and 6.25 mm ventral from dura, with the electrode angled toward the midline 20 degrees from vertical. Lesions of the DR were produced using 1.0 mA delivered through the electrode

for 6 seconds on each side. Coordinates for the PS were 0.7 mm anterior, 2 mm lateral, and 5.5 mm ventral from dura at 15 degrees from the vertical. Lesions of the PS were produced by passing 1 mA through the electrode for 10 seconds on each side. For this experiment, 20 rats received lesions in the region of the DR, 7 rats received sham DR lesions, 12 rats received lesions in the region of the PS, and 7 rats received sham PS lesions.

All animals were tested on the plus-maze and shock-probe tests according to the schedule outlined in Experiments 2 and 3. Following behavioral testing, animals were sacrificed for histological verification of lesions. As in Experiment 2, tissue slices were stained with thionin cell stain. Histological verification of DR lesions were performed using the criteria outlined in Experiment 3 for DR lesions. The area of the PS was determined to extend from 1 mm anterior to bregma to 0.3 mm from bregma, under the corpus callosum, above the nucleus accumbens, lying between the lateral ventricles. Lesions of the PS were included in the positive control group if PS lesions were complete or nearly complete (aprox. 80%), with limited extra-septal damage (within 0.5 mm of the target). Blind analysis of lesion placements confirmed nine acceptable DR lesions and seven acceptable PS lesions according to these criteria (see Figure 11). Only data from animals with confirmed DR or PS lesions , and from the sham-lesioned control animals, were

included in the statistical analysis. For statistical analysis, the sham-lesioned animals (both DR and PS) were combined into one sham group if preliminary t-tests showed no differences between the two individual groups.

Results & Discussion

The results of this experiment suggest that the DR does play a role in mediating anxiety-related behaviour in the rat. Additionally, the PS lesions produced results consistent with previous research (Gray et al., 1981; Treit & Pesold, 1990). In the plus-maze test, unweighted means ANOVA revealed robust between group differences for both percent time in, and percent entries into, the open maze arms (for % time, $F(2,26)=9.50$, $p< .005$; for % entries, $F(2,26)=8.75$, $p<.005$, see Figure 9). Subsequent Newman-Keuls post-hoc tests revealed that compared to the combined sham group , DR lesions ($p<.05$) and PS lesions ($p<.01$) produced significant increases in both measures of open-arm activity in the plus-maze.

A significant difference between groups for total number of entries into any arm was also confirmed ($F(2,26)=4.76$, $p<.05$). Post-hoc analysis revealed this difference to be a result of a significant decrease in total entries made by the PS group compared to the DR group ($p<.05$). A significant difference in the number of entries into open arms ($F(2,26)=4.55$, $p<.05$) was due to a significant increase in DR group entries compared to the

sham group ($p<.01$). A significant difference in the number of closed arm entries ($F(2,26)=7.1$, $p<.01$) was the result of a significant decrease in the number of closed-arm entries made by the PS group compared to either the DR group ($p<.01$) or the combined sham group ($p<.05$). Overall, the DR group showed a selective increase only into open arms, while the PS group was not significantly different from shams in open arm entries, but significantly reduced in closed arm entries, resulting in the significant increase in percent open arm entries. Nevertheless, both groups showed similar increases in the percent time spent in the open maze arms.

ANOVA revealed significant group differences in the time spent burying the probe ($F(2,26)=4.70$, $p<.05$, see Figure 10). Post-hoc analysis revealed that the PS group buried significantly less than the combined sham group ($p<.05$). Surprisingly, the DR group also showed a significant decrease in burying compared to shams ($p<.05$). These results suggest that procedural factors did not account for previous failures to demonstrate a significant suppression of burying after 5-HT disruption. No other shock-probe measure varied significantly between groups ($p>.05$ for immobility, number of shocks received, and reactivity to shock; see Table 5).

The histological analysis revealed that in the animals with acceptable DR lesions, there were a large number of complete DR lesions (i.e., both anterior and posterior

regions) and extra-raphe damage was minimal. Lesions of the PS were also within the outlined criteria. Although few lesions were complete, extra-septal damage was confined to the fornix.

In summary, both the DR and PS groups in this experiment showed significant anxiolytic profiles in both the plus-maze and shock-probe tests.

Figure 9: Mean (+/- SEM) percent time (open bars) in, and percent entries (hatched bars) into the of open arms of the plus-maze test by sham-, DR-, and PS-lesioned rats in Experiment 4.

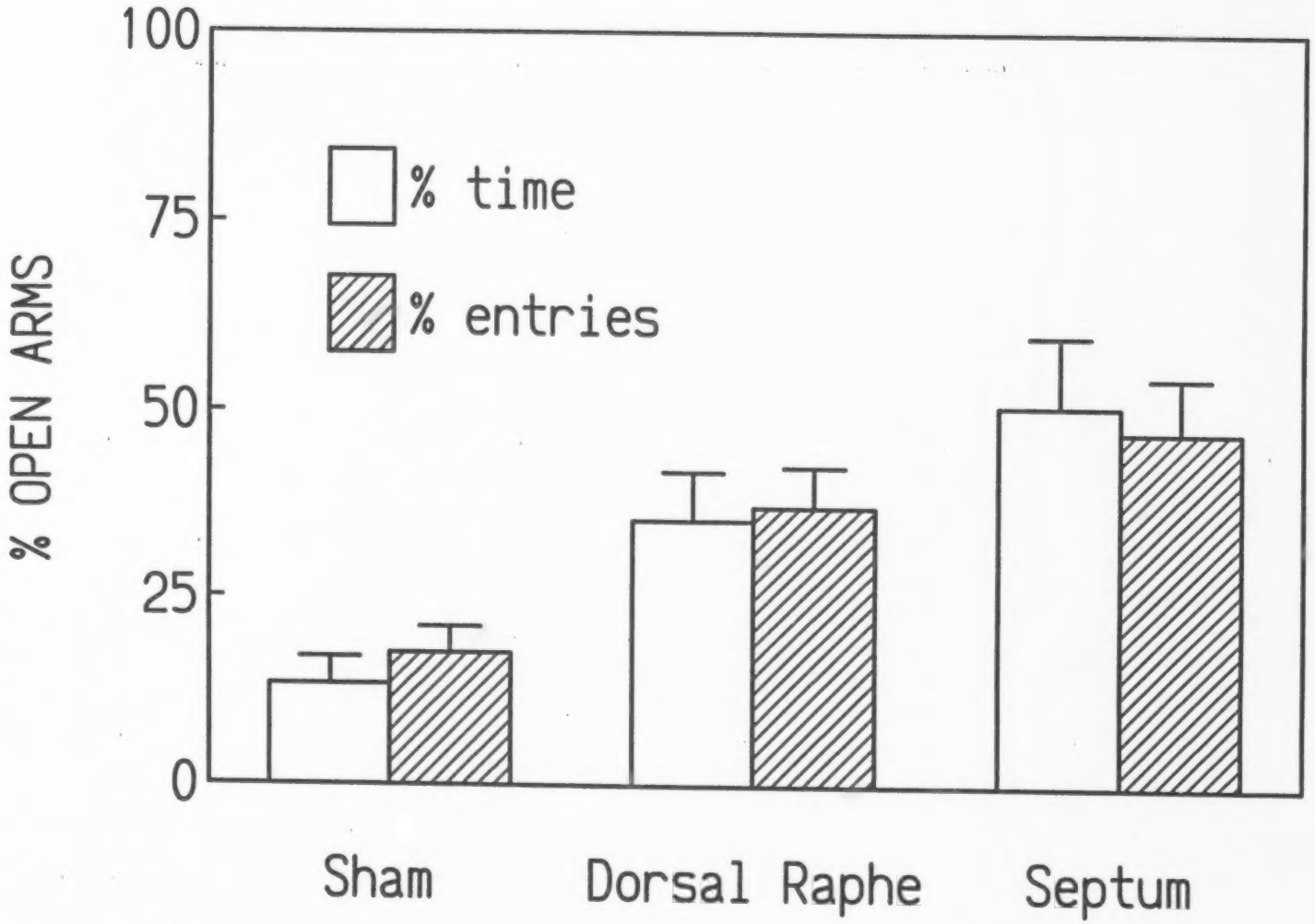

100
75
50
25
0
% OPEN ARMS
% time
% entries
Sham
Dorsal Raphe
Septum

Figure 10: Mean (+/- SEM) duration of time spent (in seconds) burying the probe in sham-, DR-, and PS-lesioned rats in Experiment 4.

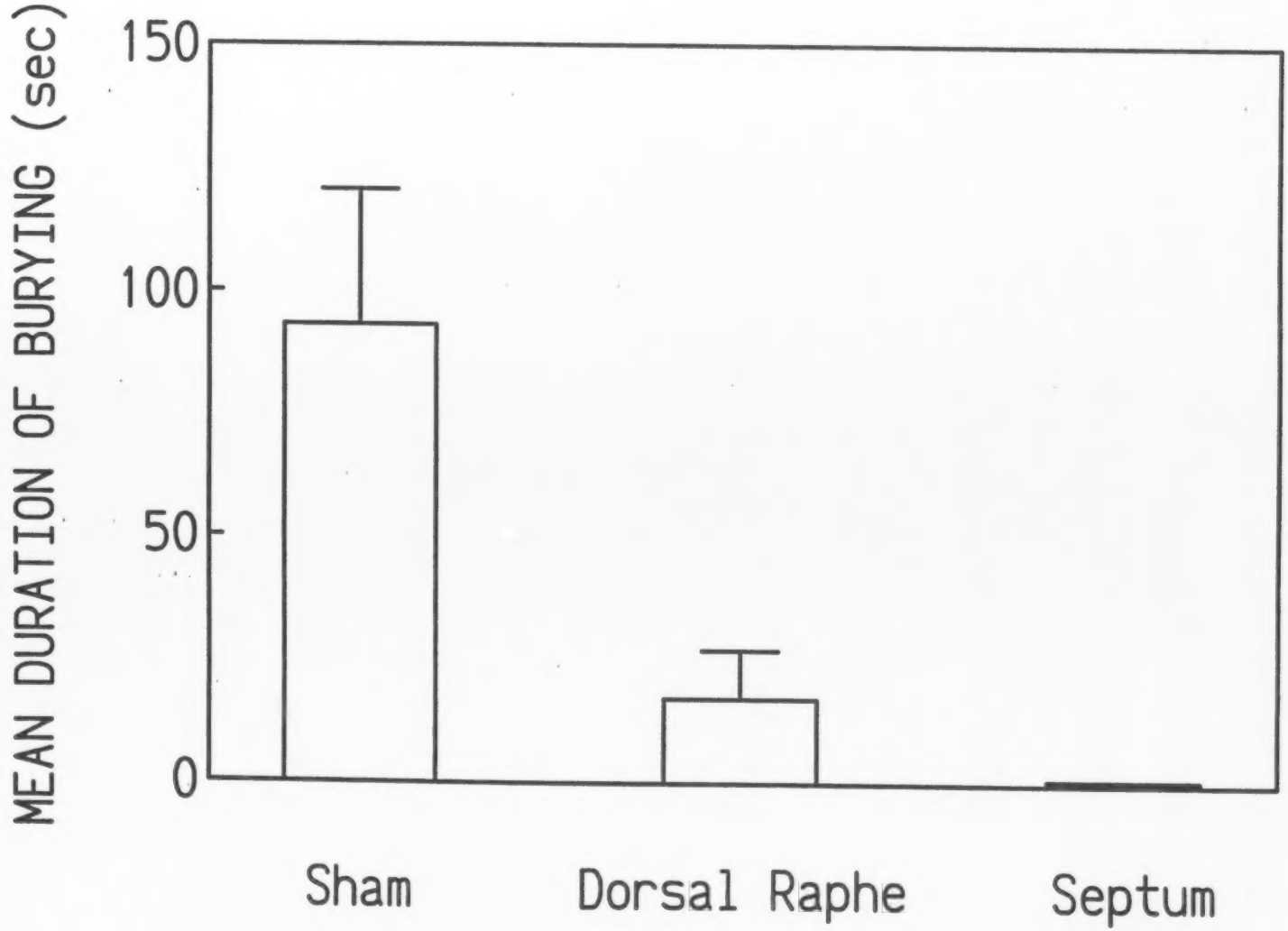
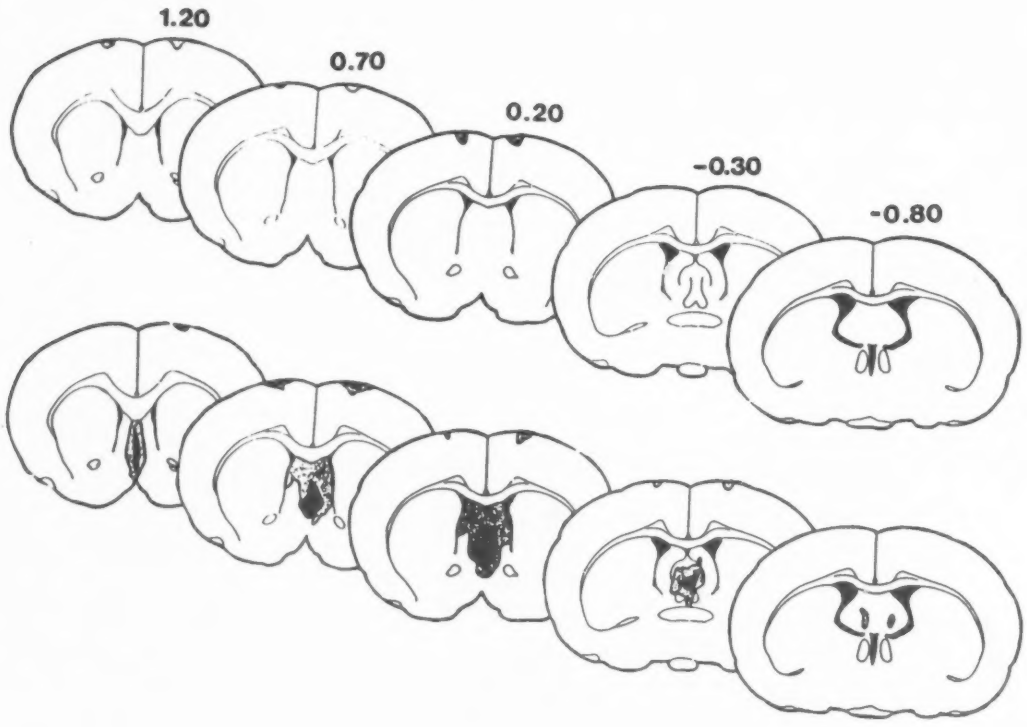

MEAN DURATION OF BURYING (sec)
150
100
50
0
Sham
Dorsal Raphe
Septum

Figure 11: Histological representation of sham PS lesions (top A), PS lesions (bottom A), sham DR lesions (top B), and DR lesions (bottom B). Light shading represents the maximum extent of any lesion, while dark shading represents a typical lesion. Numbers above each section indicate distance (in mm) from bregma.

A



B

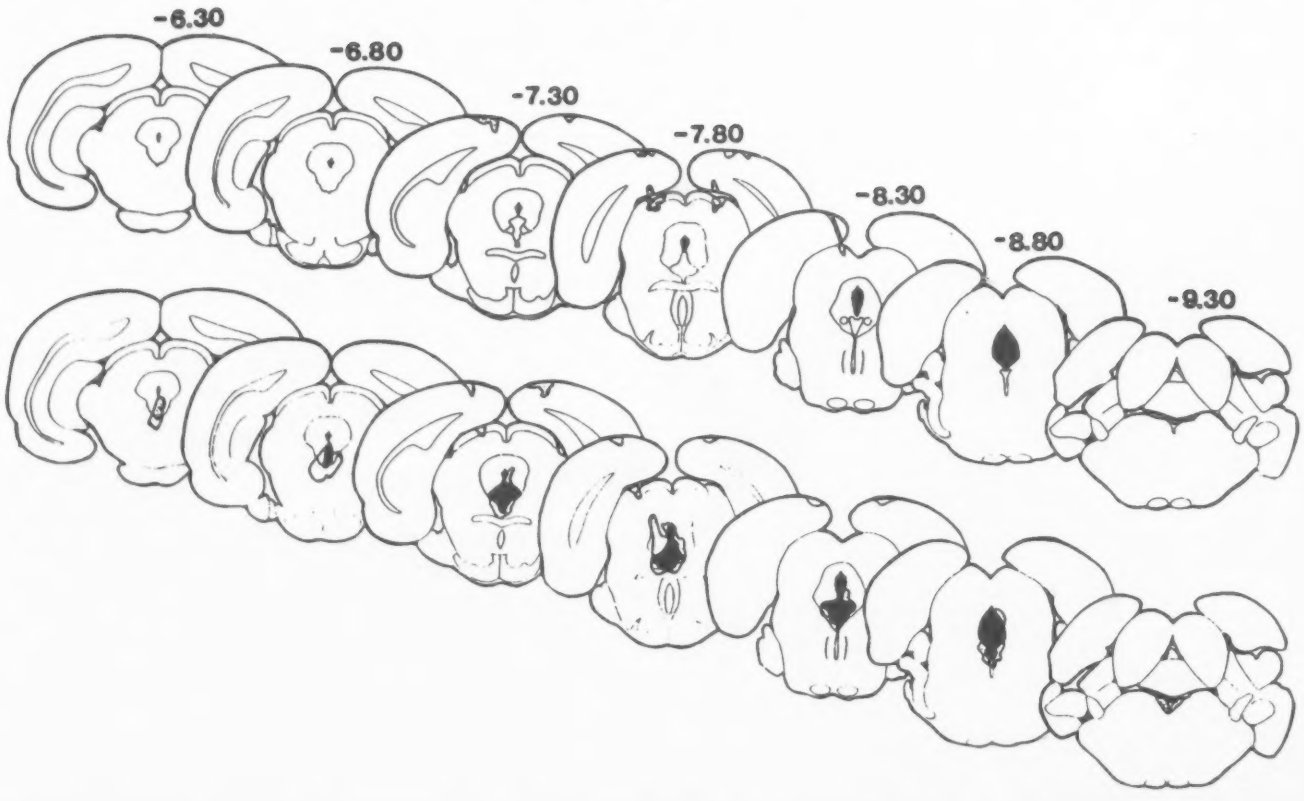


Table 5: Mean (+/- SEM) values for total-arm entries, open-arm entries, closed-arm entries, immobility, probe contacts, shock reactivity, post-hoc comparisons, and Newman-Keuls Q values for rats in Experiment 4.

Measure	Group	Mean	SEM
Total # entries into maze arms	PS	7.4	1.40
	DR	12.6	1.10
	SHAM	10.2	0.97
Entries into open arms	PS	3.2	0.64
	DR	5	1.00
	SHAM	2	0.42
Entries into closed arms	PS	4.1	1.10
	DR	7.6	0.58
	SHAM	8.2	0.72
Immobility	PS	170.1	112.6
	DR	134.2	84.6
	SHAM	44.4	21.5
# Shocks taken	PS	1.42	0.29
	DR	1.78	0.28
	SHAM	1.30	0.13
Shock reactivity	PS	2.28	0.34
	DR	2.11	0.26
	SHAM	1.77	0.20

Measure	Comparison	Newman-Keuls Q	DF	p val
% time in open arms	PS > SHAM	Q=37.5	(26)	<.01
	DR > SHAM	Q=22.2	(26)	<.05
% entries into open arms	PS > SHAM	Q=29.8	(26)	<.01
	DR > SHAM	Q=19.7	(26)	<.01
Total arm entries	PS < DR	Q=5.10	(26)	<.05
Entries into open arms	DR > SHAM	Q=3.00	(26)	<.01
Entries into closed arms	PS < DR	Q=4.09	(26)	<.01
	PS < SHAM	Q=3.41	(26)	<.05
Burying	PS < SHAM	Q=93.2	(26)	<.05
	DR < SHAM	Q=76.2	(26)	<.05

GENERAL DISCUSSION

The results of the four experiments presented in this thesis suggest that disruption of 5-HT function can produce anxiolytic-like effects in the elevated plus-maze, and to a lesser degree, in the shock-probe burying test. In Experiment 1, 5-HT was depleted using the 5-HT synthesis inhibitor, p-CPA. Compared to saline-injected control rats, rats treated with p-CPA showed significant increases in open-arm activity in the plus-maze test, and a substantial, but non-significant decrease in burying behaviour in the shock-probe test. These effects are similar to those produced by anxiolytic drugs in these same tests. In Experiments 2, 3, and 4, 5-HT function was disrupted in the brain using electrolytic lesions of 5-HT-containing cell bodies of the midbrain raphe nuclei. Compared to sham lesions, destruction of the dorsal raphe (Experiments 3 & 4), or of the dorsal and median raphe combined (Experiment 2), produced significant anxiolytic-like increases in open-arm activity in the plus-maze. Lesions of the median raphe alone (Experiment 3) did not produce a significant anxiolytic effect in the plus-maze test. In the shock-probe test, combined DR and MR lesions produced a non-significant decrease in probe burying in Experiment 2, but in Experiment 3, DR lesions alone had no effect on burying, and MR lesions actually increased (non-significantly) the duration of

burying. In spite of these inconsistencies, DR lesions in Experiment 4 produced significant anxiolytic effects in the shock-probe test.

The results of these four experiments provide some support for the view that 5-HT disruption produces anxiolysis in animals. Although earlier research utilizing the conflict model also suggested that 5-HT disruption could produce anxiolytic-like effects in rats (see introduction), these effects were often inconsistent, and were confounded with the effects of 5-HT depletion on thirst, hunger, and possibly learning mechanisms. It was argued that the use of simpler models of experimental anxiety, based on animals' untrained fear reactions to aversive (painful or novel) stimuli, might provide clear, consistent evidence that 5-HT disruption produces anxiolytic-like effects. Both the plus-maze test and the shock-probe test are based on such untrained fear reactions, are sensitive to anxiolytic drugs, and do not involve food or fluid reward. The combined use of these two tests was particularly important, since the index of anxiolysis in each test is distinct: the index of anxiolysis in the plus-maze test is an increase in behaviour (open-arm activity), while the index of anxiolysis in the shock-probe test is a decrease in behaviour (probe burying). Thus, it was argued, if anxiolytic profiles were observed in <u>both</u> tests after 5-HT manipulations, the results would be difficult to explain in terms of non-specific effects on

general activity, arousal, or behavioral inhibition. However, this ideal pattern of results was only approximated in the present experiments. Although 5-HT-depleted rats showed a reliable increase in open-arm activity in the plus-maze test, they did not show reliable and consistent decreases in burying behaviour in the shock-probe test. This inconsistency in the overall pattern of results is problematic for the hypothesis that anxiety reduction is mediated by 5-HT reduction. In the following sections, the inconsistencies in the experimental results will be discussed in more detail, and alternative interpretations of the data will be presented.

Once again, with the exception of localized MR lesions, every manipulation used to reduce 5-HT function (p-CPA, lesions of both DR and MR, or of the DR alone) produced a clear and significant anxiolytic profile in the plus-maze test. By itself, this set of plus-maze data suggests that 5-HT reduction produces robust anti-anxiety effects in rats. In contrast, animals with complete DR lesions in Experiment 4 showed a significant reduction in burying behaviour in the shock-probe test. Animals given combined DR and MR lesions (Experiment 2), or p-CPA treatment (Experiment 1), showed non-significant decreases in burying behaviour. Animals with lesions of the DR in Experiment 3 showed no change in burying behaviour, and animals with MR lesions showed non-significant increases in burying behaviour.

There are a number of possible factors which could have contributed to these inconsistencies. One factor which could have contributed, was differences in the baseline variability inherent in the two tests. In general, the plus-maze test elicits fairly stable baselines of responding, with control animals showing a mean open-arm activity level between 10-20 percent. In addition, the variability (SEM) of open-arm activity within a group is usually less than 10% (see Figures 1, 3, 6, and 9). In contrast, the mean duration of burying exhibited by control animals in the shock-probe test can be quite variable (eg., from 50 seconds in Experiment 3, to 126 seconds in Experiment 2) and the variability (SEM) within groups is usually 30 seconds or more (see Figures 2, 4, 7, and 10). Since defensive burying behaviour is a somewhat more variable measure than open-arm activity, it is possible that the shock-probe test would be less sensitive to changes in 5-HT levels than the plus-maze test. If this were the case, we might expect to see a more pronounced, significant pattern of results after 5-HT disruption in the plus-maze test than in the shock-probe test. The results of the present experiment confirm this expectation. The plus-maze test produced consistent, significant anxiolytic-like effects in nearly all cases of 5-HT depletion, while the shock-probe test produced non-significant anxiolytic trends in most cases, and a significant anxiolytic effect in only

one case (DR lesions in Experiment 4).

In spite of possible differences between the plus-maze test and the shock-probe test in terms of baseline variability, both tests appear to be equally sensitive to the effects of anxiolytic drugs. In fact, the anxiolytic effect of diazepam has been detected in the shock-probe test at doses as low as 0.2 mg/kg (Rohmer, Di Scala, & Sandner, 1990), whereas slightly higher doses (1.0 mg/kg) are required to produce anxiolysis in the plus-maze test (Pellow et al., 1975). Considering this, it does not seem likely that two tests almost equally sensitive to the effects of diazepam, would be differentially sensitive to the "anxiolytic" effects of 5-HT depletion.

Another possibility is that some secondary 5-HT mediated effect might have counteracted the expression of anxiolysis in the shock-probe test, but not in the plus-maze test. Disruption of 5-HT in the brain has been shown to alter pain sensitivity in animals, generally increasing the sensitivity to pain (Hamon et al., 1990). In addition, 5-HT neural systems appear to be involved in inhibiting a pain pathway to the thalamus (e.g., Preito-Gomez et al., 1989). Considering this, it is likely that shock-motivated models of anxiety, such as the shock-probe test, might be complicated by 5-HT-induced changes in pain sensitivity, while models which do not employ painful stimuli, such as the plus-maze test, would not be affected. Perhaps the

failure of the shock-probe test to reveal consistent anxiolysis was a result of a concurrent increase in pain sensitivity, which masked a 5-HT reduction-induced anxiolytic effect. However, if increased pain sensitivity were responsible for antagonizing the anxiolytic effects of 5-HT manipulations, it might be expected 5-HT-depleted animals would either be more reactive to shock, would take fewer shocks, or might possibly even bury the probe longer than normal animals.

The results from the shock-probe test are clearly not consistent with these expectations. Animals treated with p-CPA (Experiment 1) did not show significant differences compared to saline-injected controls in their reactivity to shock, or in the number of probe contacts made, but did show a reduction in their duration of burying (see Figure 1 & Table 2). Similar results were found in animals with combined lesions of the DR and MR (see Table 3). In Experiment 3, DR-lesioned animals showed a decrease in shock reactivity, but no suppression of burying, and no significant difference in the number of probe contacts made. In Experiment 4, animals sustaining DR lesions showed a significant suppression of burying, but no difference in shock reactivity or number of probe contacts. In none of the four experiments in this thesis was there direct evidence of increased pain sensitivity in 5-HT depleted animals. In order to assess whether increased pain

sensitivity indeed acts to counteract the suppression of the burying response in 5-HT-disrupted animals, it would be necessary to investigate pain sensitivity in independent tests of analgesia (i.e., the tail-flick test).

Changes in general activity induced by 5-HT disruption might also introduce variability into the behavioral data, theoretically either mimicking or masking the expression of an anxiolytic profile in rats. Hyperactivity has often been observed in animals after 5-HT disruption (e.g., Fibiger & Campbell, 1971; Marsden & Curzon, 1977). Casual observation in the present experiments revealed that animals given p-CPA, MR lesions, or combined MR and DR lesions, did exhibit some general behavioral agitation in their home cages, whereas animals given DR lesions did not. These observations are compatable with evidence suggesting hyperactivity is due to disruption of 5-HT in the MR (Azmitia, 1975; Kostowski, Giacalone, Garattini, & Valzelli, 1968). Despite these observations, however, no other evidence of hyperactivity was observed during handling of rats in the present experiments, and more importantly, no hyperactivity was seen in either the plus-maze or the shock-probe tests of anxiolysis. Increases in the number of arm entries in the plus-maze were seen after 5-HT disruption, but this increased activity was always selective for the open arms of the maze.

In the case of the shock-probe test, hyperactivity

could theoretically either potentiate or interfere with the burying response. However, the only significant change in general activity seen in this test occurred in Experiment 1. Rats treated with p-CPA showed a significant decrease in activity, which was correlated with with a (non-significant) decrease in burying. In this case, the decrease in burying behavior might have been secondary to an overall decrease in general activity. In any case, there was no evidence that hyperactivity affected animal behaviour in these tests.

Alternatively, it is possible that some of the variability in the effects seen in the plus-maze and the shock-probe tests was related to an effect of 5-HT disruption on the ability of animals to inhibit responding. There is some evidence which suggests that low brain levels of 5-HT might be related to increased agressiveness in rhesus monkeys, while high levels of 5-HT are related to timidity (Higley et al, 1990). Additionally, in a standard operant paradigm, p-CPA administration increased resistance to extinction at the end of a continuous reinforcement schedule in rats (Rosen & Freedman, 1974). Furthermore, electrolytic lesions of the raphe in rats impaired performance on a schedule of reinforcement for low response rates (Wing & Wirtshafter, 1982). These studies suggest 5-HT depletion might disrupt animals' ability to inhibit responses that are no longer rewarded, or responses that lead to reward reduction. It has been proposed that this

"disinhibition" of behaviour is the source of what appears to be anxiolysis in conflict tests, where animals must inhibit punished responding (Soubrie, 1986). It is possible that general "disinhibition" induced by 5-HT reduction could have contributed to the results seen in the plus-maze test in the present experiments. If the inhibition of open-arm exploration in normal animals is maintained by 5-HT, animals with reduced 5-HT function would be expected to show a "disinhibition" of open-arm exploration. The results of the present experiments are in fact consistent with this explanation.

Despite this, the concepts of "conflict" and "disinhibition of behaviour" cannot be easily applied to the shock-probe test. To begin, defensive burying is not inhibited by the "fearful" shock stimulus. Instead, it is elicited by the shock. It is therefore difficult to see how disinhibition applies to this measure of anxiety. Any factor which produces a general behavioral "disinhibition" would not necessarily be expected to alter the burying response. In fact, with one exception, defensive burying was not significantly affected by the 5-HT manipulations used in these experiments. In a sense, this dichotomy of results in the plus-maze and shock-probe tests after 5-HT depletion, favors the hypothesis that 5-HT reduction produces a general response disinhibition: Apparent "anxiolytic" effects were reliably observed in the plus-maze

test as an increase in (inhibited) open-arm activity, but were not reliably observed in the shock-probe test as a decrease in (non-inhibited) burying.

However, some data from the shock-probe test are inconsistent with the "disinhibition" interpretation. A disinhibited animal might be expected to approach the probe more often, and thereby receive more shocks. The shock-probe test data do not support this expectation. None of the 5-HT depleted rats showed an increase in the number of shocks received, except animals with MR lesions (Experiment 3), and these experimental animals were the only ones that did not show any significant increase in open-arm activity in the plus-maze test. Thus, while it is possible that "general disinhibition" might be related to some of the variability in results seen in the two tests of anxiolysis in these experiments, this idea cannot account for the entire pattern of results.

It is possible that the effects of 5-HT depletion on animals' learning ability might influence animal responding in the shock-probe test. Electrolytic lesions of the raphe have been shown to impair learning of a one-way avoidance response (Hole & Lorens, 1975), and to impair stimulus discrimination ability in rats (Wirtshafter et al., 1983). Administration of p-CPA has been shown to disrupt latent inhibition in rats (Solomon, Kiney, Scott, 1978). In the plus-maze test of anxiolysis, animals avoid the open maze

arms without any prior experience or training, and do not need to learn any associations between stimuli and responses. In the shock-probe test, animals bury the shock source without prior training or experience, but some learning of an association between the probe and electric shock must occur before animals can accurately direct the burying response toward the probe (Pinel & Treit, 1978). Thus, neural manipulations that disrupt associations of stimuli with aversive consequences might be expected to disrupt burying behaviour directed toward the probe.

In the four experiments discussed in this thesis, most 5-HT depleted rats showed non-significant decreases in the duration of burying. While this trend is consistent with a disruption in associative learning ability in these animals, there are two problems this interpretation. First, other studies (e.g., Hole & Lorens, 1975) investigating raphe lesion effects on learning ability suggest that MR-lesioned animals have the greatest learning deficits. However, the results of the present experiments show that MR-lesioned animals actually buried the probe more, not less, than control animals. Second, with one exception, 5-HT-depleted animals were able to avoid contact-induced shocks from the probe at least as well as control animals. This would not be expected if these aimals were unable to learn an association between the shock and the probe. Thus, it is unlikely that 5-HT depletion produced the behavioral effects

seen in the shock-probe tests through a disruption of associative learning.

Although it has been assumed that the behavioral effects seen in these experiments were due to a disruption of 5-HT, it is possible that some of these effects were a result of alterations of neurotransmitters other than 5-HT. However, histological analysis of raphe-lesioned animals revealed no damage to the locus ceruleus or substantia nigra, areas containing high densities of catecholamine neurotransmitters (NE and DA, respectively). While it is possible that electrolytic lesions of the raphe might have marginally or indirectly affected NE or DA, the behavioral results from these experiments were not due to direct damage to the locus ceruleus or substantia nigra. Furthermore, the behavioral effects of electrolytic lesions of the raphe generally paralleled the behavioral effects of p-CPA treatment, which produces a very selective depletion in 5-HT when administered under the dose schedule used in Experiment 1 (Koe & Weissman, 1966). For these reasons, it is unlikely that catecholamine disruption can account for the behaviours seen in these experiments. Nevertheless, definitive answers to these questions must await similar studies which include concurrent brain assays of neurotransmitter systems.

5-HT depletion and anxiolysis: Regional specificity. If the magnitude of anxiolysis in animal models was inversely proportional to the magnitude of 5-HT depletion in the brain, we might expect that p-CPA treatment would produce the most dramatic anxiolytic effects in rats, followed by combined DR and MR lesions, and then by individual lesions of either the DR or the MR. This theoretical pattern of results is incongruent with the results from the present experiments, however. In fact, it could be argued that 5-HT in the DR was critical in mediating experimental anxiety in the present experiments. Although previous research suggests that electrolytic lesions of either the DR or the MR will reduce total forebrain 5-HT to a similar extent in rats (Jacobs et al., 1974), DR (and not MR) lesions in Experiment 3 produced significant anxiolysis in the plus-maze test, and in Experiment 4, DR lesions produced clear anxiolysis in both the plus-maze and the shock-probe tests. Furthermore, there was some histological evidence that more complete DR lesions were associated with more complete anxiolytic effects. In contrast, electrolytic lesions of the MR failed to produce clear evidence of anxiolysis in either the plus-maze or the shock-probe tests.

The MR has been shown to project to the DR (Aghajanian & Wang, 1978). Since there are 5-HT autoreceptors in the DR which inhibit 5-HT release (Moret, 1985), these MR connections may serve to inhibit the DR, reducing 5-HT

transmission to the forebrain. If this is the case, it seems reasonable that once the inhibitory influence of the MR is removed, the resultant release of DR inhibition might actually increase anxiety by making more 5-HT available to the forebrain. In other words, MR lesions might be "anxiogenic" rather than anxiolytic. It is important to note, however, that although MR-lesioned rats in Experiment 3 showed an increase in burying compared to controls, they did not show a corresponding anxiogenic decrease in open-arm activity in the plus-maze test. Further investigation of the effects of MR lesions on animal "anxiety" is necessary before any conclusions can be made regarding it's role in mediating experimental anxiety. However, it is possible this factor contributed to some of the variability seen in this set of experiments.

Although it has been argued that the DR (and not brain 5-HT in general) is critical in mediating experimental anxiety, there is the problem of the discrepancy between the results obtained after DR lesions in Experiment 3 and in Experiment 4. Lesions of the DR in Experiment 3 produced a significant increase in the percent time in the open arms of the plus-maze, but only a non-significant increase in the percent of open-arm entries. In the shock-probe test, lesions of the DR in Experiment 3 failed to produce any effect on the duration of burying, but lesions of the DR in Experiment 4 produced robust and significant anxiolytic

effects on defensive burying in the shock-probe test, and on both measures of open-arm activity in the plus-maze test. If the DR-lesioned groups in Experiment 3 and 4 received the same lesion, behavioral data should not have differed so greatly between these experiments. However, close inspection of the histologies from the two DR-lesioned groups showed that they did not sustain an equivalent degree of damage to the target site. There were several differences between DR lesions in Experiment 3 and those in Experiment 4. Although both DR groups met the criteria for an acceptable DR lesion, animals with DR lesions in Experiment 3 appeared to sustain more extra-raphe damage within 0.5 mm of the DR than did animals with DR lesions in Experiment 4. More importantly, however, was the difference in the amount of DR tissue actually lesioned. In Experiment 3, animals tended to sustain lesions that spared much of the anterior portion of the DR. Failure to lesion the entire DR, especially the anterior portion spares much of the 5-HT transmission to the forebrain (Lorens & Guldberg, 1974). In Experiment 4, however, the DR lesions were usually complete. Considering this difference in the DR lesions in these experiments, it is possible that the difference in degree of anxiolysis seen in these animals might reflect differences in the degree of 5-HT disruption in the DR. This idea suggests that 5-HT in the DR mediates "anxiety" in animals. Nevertheless, although animals with

more complete lesions of the DR in Experiment 4 showed more dramatic anxiolytic behavioral profiles in both tests of experimental anxiety, animals with complete lesions of the DR (combined with MR damage) in Experiment 2, showed significant anxiolysis in only the plus-maze test, and not in the shock-probe test. This inconsistency is difficult to reconcile with the hypothesis that complete DR lesions lead to complete anxiolysis, although it is possible that the additional damage to the MR in Experiment 2 may have weakened the effects of specific damage to the DR.

The septum-DR link. In spite of the complications discussed previously, the hypothesis that 5-HT disruption leads to anxiolysis in animals has some degree of support, especially from the plus-maze test. While there are still a great number of questions left to be answered, this finding might not be surprising, given that the raphe nuclei send 5-HT projections to a variety of limbic structures, which have long been implicated in emotional behavior (Papez, 1937). These limbic areas include the septum, amygdala, hippocampus, and nucleus accumbens (Consolazione & Cuello, 1982; Gray, 1982; Parent et al., 1981). While 5-HT input into any of these areas might modulate anxiety in animals, the behavioral effects of the posterior septal lesions found in Experiment 4 suggest the connections of the raphe with the septum might be particularly important. Lesions of the

septum have previously been shown to produce anxiolysis in the plus-maze and shock-probe tests (Gray et al., 1981; Treit & Pesold, 1990; Pesold & Treit, 1992). In Experiment 4, lesions of the either the PS or the DR produced similar anxiolytic effects in both tests. This similarity in the behavioral effects of DR and PS lesions may be related to the well known anatomical connections between the DR and the septum (Segal & Landis, 1974). Furthermore, serotonergic neurotoxic lesions of the septum using 5,7-DHT produced anxiolytic-like effects in a social interaction test of anxiolysis (Clarke & File, 1982). In combination, this evidence suggests 5-HT input into the septum might modify anxious behaviour in rats. This evidence is consistent with Gray's (1982) notion that the raphe-septo-hippocampal system of the brain is central to producing anxiety in animals.

It is interesting to note that PS lesions completely eliminated defensive burying in Experiment 4, whereas DR lesions suppressed but did not eliminate this response. This might suggest that the PS is central to anxiolysis in the shock-probe model, and that 5-HT projections from the DR modify the expression of this fear reaction through the septum. It would be interesting to explore this possibility by assessing the effects of 5,7-DHT lesions of the septum in the plus-maze and shock-probe tests.

5-HT and GABA. A final point concerns the possible connection between 5-HT, GABA, and anxiety. There is a great deal of evidence that benzodiazepine anxiolytics exert their behavioral effects through facilitation of GABA neurotransmission in the brain (e.g., Haefely, 1989; Hommer, Skolnick, & Paul, 1987). There is also evidence that GABA stimulation inhibits DR firing in the brain (Gallager, 1978) Manipulations using GABAergic drugs or benzodiazepines in the DR also modify anxiety in animals (Collinge, Pycock, & Taberner, 1983; Green et al., 1983; Higgens et al, 1988; Sainati & Lorens, 1982; Soderpalm & Engel, 1989; Soderpalm & Engel, 1991). It is therefore possible that BZs exert their anxiolytic effects indirectly through an inhibition of 5-HT transmission as a result of facilitating GABA. If this is the case, it seems understandable that either BZ-induced increases in GABA transmission, or direct decreases in 5-HT transmission might produce anxiolysis in rats.

Conclusions. Although these speculations are interesting, none of them offer convincing explanations of the entire pattern of results obtained in the present experiments. The simple idea that a reduction of 5-HT should uniformly lead to a reduction in experimental anxiety was not confirmed by the results of the present experiments. Although the results from the plus-maze test were in agreement with the 5-HT hypothesis, the results of the shock-probe tests were

often inconsistent with this hypothesis.

Thus, the inconsistencies which plagued the previous literature (see introduction) also marred the results of the present experiments. Clearly, further research is needed to show that 5-HT plays a direct and pervasive role in modulating experimental anxiety. Nevertheless, the elevated plus-maze test and the shock-probe burying test appear to be useful models with which to explore the relationship between 5-HT and anxiety.

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